Managing forb diversity in temperate grassy ecosystems

David Paroissien Johnson

BEd, GCEMD, MEnv (Research)

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Declaration by author

This thesis contains no material that has been accepted for the award of any other degree or diploma in any university. To the best of the author's knowledge and belief it contains no material previously published or written by another person, except where reference is made in the text or chapter statements of contribution.

David Johnson

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At this point I must pay tribute to Chris McElhinny, a brilliant teacher who is almost single-handedly responsible for inspiring me to consider doing some environmental research. His "Ecological restoration and management" course really captured my imagination, but sadly, Chris was not able to supervise my Master's research due to illness. I am deeply indebted to Phil Gibbons for, not only agreeing to supervise my master's research in Chris' place, but also for facilitating a research scholarship for my Master's research–offered by the Australian Capital Territory (ACT) Government under an offset obligation–and later for encouraging me to consider applying for the follow-on scholarship for PhD research under the same offset agreement. I cannot imagine how my life would be if not for the inspiration and mentorship I was so fortunate to receive from Chris and Phil.

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the important role my whole family has played in endowing me with a fundamental love of the land, the desire to understand nature and learn how to live with it sustainably and the resilience needed to finally reach the end of my PhD. Much of the inspiration to study the environment, and my general make-up as a person, for which I am eternally grateful, is a product of the influence of my wonderful mother, Marcia “Possie” Johnson, who started planting native species more than 50 years ago, long before restoration was a thing. Sadly, you aren’t here to see it, but my thesis is dedicated to you Possie.

Papers forming the thesis by compilation

This thesis is a Thesis by Compilation, as set out in ANU's *Higher degree by research - thesis by compilation and thesis by creative works procedure* (https://policies.anu.edu.au/ppl/document/ANUP_003405).

The thesis was conceived, developed, and written by the candidate David Johnson with advice from Philip Gibbons, Jane Catford and Don Driscoll. Chapters Two, Three, Four and Five of this thesis correspond to the following papers listed below. All collaborating authors agree to the inclusion of papers listed below and agree to the description of their contribution to papers (where applicable).

Chapter One
Introduction

Chapter Two
A comparison of native and exotic forb responses to eight variables that influence forb habitat quality in temperate grasslands

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Authors: **David P. Johnson,** Jane A. Catford, Don A. Driscoll, Wade Blanchard, John Gillen, Philip Gibbons
Status: Submitted
Journal: Journal of Applied Ecology

Contributions: DPJ, PG and JG conceived and conducted the survey; DPJ and JG designed and conducted the soil analyses; DPJ developed the concept for the paper in collaboration with JAC, DAD and PG, based on a statistical approach conceived by WB; DPJ analysed the data in consultation with WB; DPJ

wrote the paper with feedback and editorial advice from JAC, DAD and PG, reviewed by JG and WB.

Chapter Three

Fine-scale variables associated with the presence of native forbs in natural temperate grassland

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Authors: **David P. Johnson,** Jane A. Catford, Don A. Driscoll, Philip Gibbons

Status: Submitted

Journal: Austral Ecology

Contributions: PG conceived the case-control survey approach in collaboration with DPJ, JAC, DAD; DPJ designed the survey, collected and analysed the data, and wrote the paper with feedback and editorial advice from JAC, DAD and PG.

Chapter Four

Seed addition and biomass removal key to restoring native forbs in degraded temperate grassland

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Authors: **David P. Johnson,** Jane A. Catford, Don A. Driscoll, Philip Gibbons

Status: Accepted (24 October 2017, online in Early View 8 January 2018)

Journal: Applied Vegetation Science

Contributions: DPJ conceived and designed the field experiment in collaboration with JAC, DAD and PG; DPJ established the experiment, collected and analysed the data, and wrote the paper with feedback and editorial advice from JAC, DAD and PG.

Chapter Five

Restoring and maintaining forb diversity in natural temperate grassland

Authors: **David P. Johnson,** Jane A. Catford, Don A. Driscoll, Philip Gibbons

Status: Submitted

Journal: Ecological Management and Restoration

Contributions: DPJ conceived the conceptual model proposed in this paper in collaboration with PG, as a synthesis of ideas developed in collaboration with JAC and DAD; DPJ wrote the paper with feedback and editorial advice from and JAC, DAD and PG.

Abstract

Native forbs contribute significantly to grassland species diversity, with flow-on benefits for habitat value (eg. for birds, reptiles, invertebrates), ecosystem services (eg. pollinator diversity, pest control) and resistance to invasion by exotic plants. Humans have modified grasslands around the world with agricultural land use and/or poor management, often resulting in significant loss of native forb diversity. Research over several decades identifies elevated soil nutrients, exotic species and changed disturbance regimes as threats to grassland forb diversity, but despite this knowledge, forb restoration remains difficult and forb diversity in temperate grasslands remains low. My research aimed to further our understanding of forb ecology and the challenges of maintaining and restoring native forb diversity, focusing on grassy ecosystems in south-eastern Australia. The research was predominantly conducted within an area of White Box – Yellow Box – Blakely's Red Gum woodland and derived grassland in a nature reserve in Canberra, in south-eastern Australia. This ecosystem is listed as critically endangered, largely on the basis of lost ground-layer diversity.

This thesis includes an introduction (or context statement) outlining my research program and how it contributes to the literature, three chapters describing empirical research projects I conducted for the PhD, and a final chapter representing a synthesis of my results in the form of a conceptual model with management recommendations. Chapters two to five are written as manuscripts submitted or accepted for publication in scientific journals.

Chapter 2: A comparison of native and exotic forb responses to eight variables that influence forb habitat quality in temperate grasslands

The second chapter (submitted) is based on a survey of the study area, in which I collected data on ground-layer vegetation, soil chemistry and soil physical properties, and topography. I analysed forb responses to eight environmental variables representing landscape, soil, and vegetation biomass. I identified thresholds of grass cover, litter cover and soil phosphorus above which native forbs are much less likely to occur than exotic forbs.

Chapter 3: Fine-scale variables associated with the presence of native forbs in natural temperate grassland

The third chapter (submitted) is based on a case-control survey designed to determine the fine-scale variables associated with the presence/absence of individual native forbs. Consistent with the results from our other research, I identified that dead biomass cover, grass cover, and exotic forb cover were negatively associated with the odds that a native forb would be present in temperate grassland.

Chapter 4: Seed addition and biomass removal key to restoring native forbs in degraded temperate grassland

The fourth chapter (manuscript accepted by *Applied Vegetation Science*) is based on a controlled field experiment designed to test hypotheses arising from our previous results. I applied treatments that reduce above ground biomass (grass tussocks, leaf litter, and exotic species) and added seed of 14 native forb species. Native and exotic forb seedlings were both negatively affected by living grass cover, however, native forb seedlings were more restricted by litter than exotic forb seedlings. Our results suggest that naturally occurring seed in disturbed grassland is likely to be inadequate for restoration.

Chapter 5: Maintaining or restoring forb diversity in temperate grasslands

The fifth chapter (submitted) provides a synthesis of our PhD results as a conceptual model of habitat suitability for native forbs in relation to available phosphorus and biomass cover. I propose some thresholds for these variables and discuss appropriate restoration actions. This chapter targets practitioners aiming to conserve native diversity in temperate grasslands.

Key words

Biomass; Environmental restriction; Exotic plant invasion; Grassland diversity; Grassland management; Grassland structure; Leaf litter; Native forb; Propagule availability; Recruitment limited; Resource availability; Seed addition; Seed limited; Seedling emergence; Soil phosphorus; Temperate grassland.

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Chapter One:

Introduction

Background

Natural grasslands around the world are threatened by agriculture, urbanisation, and altered disturbance regimes (McDougall and Morgan, 2005; McIntyre, 2011; Kiehl *et al.*, 2006; Öster *et al.*, 2009; Howe, 1994). This leads to declines in both the areal extent and biodiversity of grasslands, with native forb species particularly affected (Tremont and Mcintyre, 1994; Brandt and Seabloom, 2012; Stevens *et al.*, 2010). Forbs significantly contribute to the floral diversity of grassy ecosystems (Tremont and Mcintyre, 1994; McCain *et al.*, 2010; Pallett *et al.*, 2016). Higher levels of floral diversity in grasslands are associated with higher levels of soil protection, water filtration and temporal stability in ecosystem productivity, aesthetic values, and lower levels of exotic plant invasion (Tscharntke *et al.*, 2005; Tilman *et al.*, 2006; Wratten *et al.*, 2012). Native forb diversity also provides food and habitat for other species, including those that perform ecosystem services such as pollination and biological pest control (Schmidt-Entling and Döbeli, 2009; Williams *et al.*, 2015). Restoring and maintaining forb diversity is thus a key conservation goal for grasslands around the world (Hobbs *et al.*, 2013).

The forb component of naturally diverse grasslands is negatively affected by most forms of agriculture in a number of ways (Lunt, 1997a; Kirkpatrick *et al.*, 2005; Hamilton, 2001). Firstly, cropping and sowing pastures replaces large areas of native grassland with simplified communities or monocultures. Secondly, the use of fertilisers elevates soil nutrients to a level that gives exotic annuals a competitive advantage over native forb species (Prober and Thiele, 2005; McIntyre and Lavorel, 2007; Hautier *et al.*, 2009).

Thirdly, grazing reduces forb diversity by increasing the exposure of forbs which would otherwise be sheltered between the grass tussocks, through preferential grazing by livestock, nutrient enrichment, trampling, and soil compaction (Lunt, 1997a; Kirkpatrick *et al.*, 2005). Fourthly, a lack of disturbance such as fire can lead to a build-up of excess biomass and deterioration of the open grassland gap structure, which reduces the quantity and quality of suitable habitat available for forbs to germinate and grow. The combination of these threats, and a short-lived seedbank (Morgan, 1998b), in Australian species at least, probably caused many species to become locally extinct from large areas quite rapidly (Lunt, 1996).

The lack of a persistent seedbank means that restoration projects usually require the re-introduction of species. However, in a review of plant re-introductions around the world Godefroid et al (2011) found a moderate success rate at best, and this assessment was frequently based on short-term success only, as monitoring typically stops after approximately four years. In Australia, successful restorations of self-sustaining forb populations are uncommon (Morgan and Williams, 2015; McIntyre *et al.*, 2017), even though researchers have found ways to reduce soil fertility (Prober and Thiele, 2005; Prober *et al.*, 2013), reduce competition from weeds (Gibson-Roy *et al.*, 2010; Prober *et al.*, 2004), and excess biomass (Morgan, 1998c). These techniques can all improve the ability of native forbs to establish within existing grasslands, but recruitment of new seedlings remains a problem (Morgan, 1998b).

Morgan (2001) observed very low levels of seedling recruitment, even in established populations, and concluded that many native forbs species were only able to maintain their presence from year to year though vegetative regeneration. This strategy alone will not sustain forb populations in the long term, as forb species do not usually live as long as grass species (Lauenroth and Adler, 2008), and senescing plants must eventually be replaced by seedlings if the population is to remain viable in the long-term. Lauenroth and Adler (2008) also found that most mortality within grassland species, including forbs, occurs when plants are young. Many researchers have tried to increase the number of seedlings surviving through to maturity by using various types of disturbance and techniques for managing above ground living biomass and litter accumulation (Isselstein *et*

al., 2002; Wirth and Pyke, 2003; Holl and Hayes, 2006; Smallbone *et al.*, 2007; Williams *et al.*, 2007). Grassland studies in many countries (Tremont and Mcintyre, 1994; Williams *et al.*, 2007; Eddy, 2005; Kahmen and Poschlod, 2008) have revealed similar requirements for periodic disturbance or management to control the build-up of above ground biomass in order to retain a diversity of gap-dwelling species. A meta-analysis of experiments focusing on the impacts of litter management on seedling recruitment and survival (Loydi *et al.*, 2013) found that low to medium amounts of litter can improve forb germination and seedling survival, particularly under dry conditions, but high litter loads will inhibit seedling recruitment. For these reasons, biomass and litter management are likely to be an on-going requirement for the management of productive grasslands.

To improve on the modest forb restoration success rate so far, I sought to identify the key variables determining native forb habitat quality and understand how they are changed by biomass management. My aim was to determine the conditions that define suitable habitat for native forb species as a functional group, a common approach in plant community research (Lavorel *et al.*, 2007), and not to focus on individual species which may vary in their responses. I also set out to identify differences in the way that native and exotic forbs respond to the key variables and biomass management, as actions designed to increase the richness and abundance of native forbs may inadvertently inhibit native forbs by facilitating exotic species instead (Faithfull *et al.*, 2012; Driscoll and Scheiner, 2017). The over-arching goal of my PhD research was to provide information that may help practitioners protect or restore self-sustaining populations of native forb species (Prober and Wiehl, 2011; Tognetti and Chaneton, 2015; Driscoll and Scheiner, 2017).

Research approach

Our research focused on the temperate grassland that makes up the ground-layer of rapidly declining under-storey of Box-Gum grassy woodland and derived grassland ecosystems in south-eastern Australia. Box-Gum grassy woodland and derived grassland ecosystems are listed as Critically Endangered under the Australian Government's Environment Protection and Biodiversity Conservation Act 1999 (the EPBC Act), largely because of lost forb diversity in the under-storey

(https://www.environment.gov.au/system/files/resources/be2ff840-7e59-48b0-9eb5-4ad003d01481/files/box-gum.pdf; accessed 12-10-2017). At the time of European settlement in Australia, Box-Gum grassy woodland and derived grassland spanned a large area throughout south-eastern Australia (Figure 1.1). It has been estimated that less than 0.05% of the original (pre-European) extent of this ecosystem retains an intact under-storey (Prober and Thiele, 2005). Some of the largest remaining remnants are in the Australian Capital Territory (ACT) region (Figure 1.1). The research documented in this thesis was conducted within an area of Box-Gum woodland and derived grassland in a Canberra Nature Reserve within the ACT, funded by the ACT Government under an offset obligation.

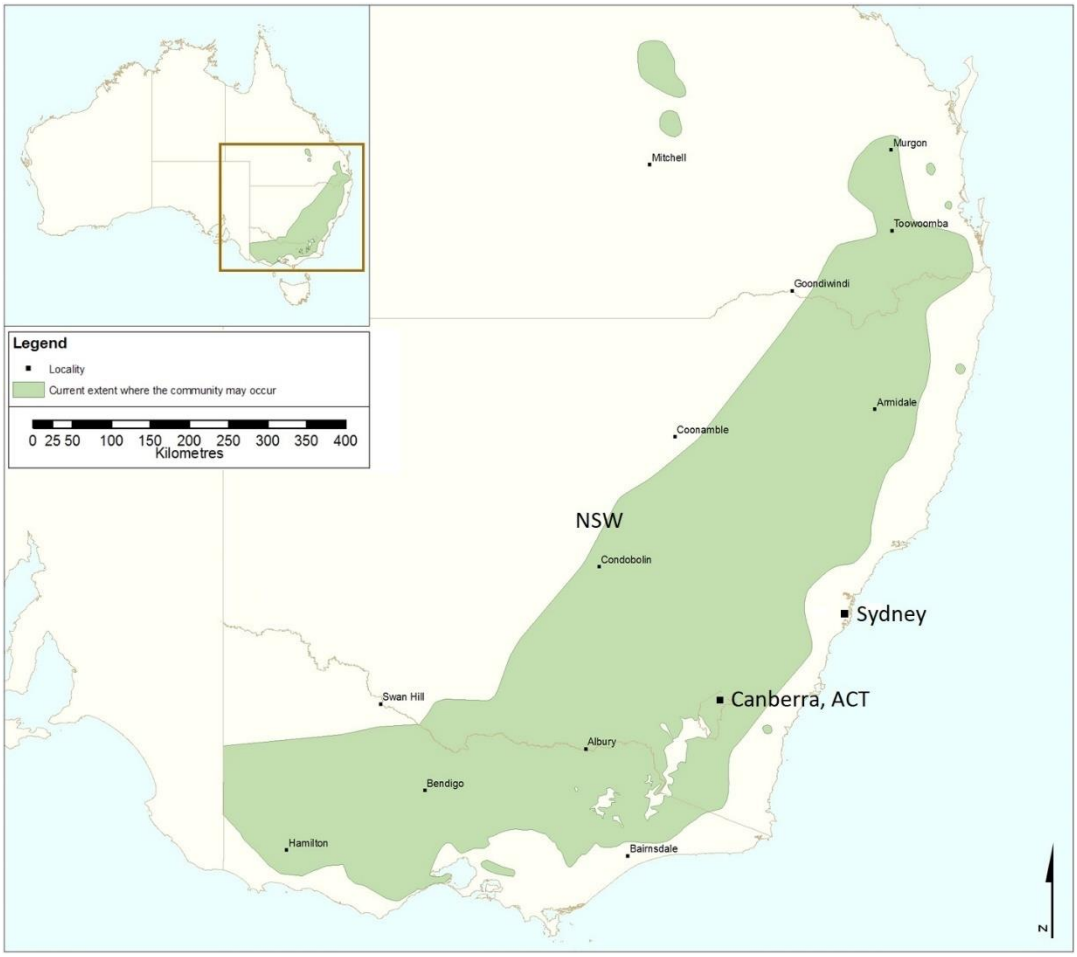

Figure 1.1. Predicted pre-European distribution of Box-Gum woodland and derived grassland. The study site is located on the edge of Canberra, ACT, in temperate south-eastern Australia. (Source: Australian Government, Department of the Environment, 2010)

In addition to information from the literature, reviewed above, I spoke with local practitioners to understand the main threats to grassland diversity and the difficulties associated with managing natural grasslands in the ACT region. The main threats identified by practitioners were consistent with the literature: soil nutrient enrichment, competition for resources associated with excessive growth in dominant grass species, and competition from exotic species.

My research approach included two complementary methods of data collection: observational survey and an experimental approach. Observational surveys were undertaken to identify the key variables associated with the presence of native forbs, one with a broad community focus and the other a narrow focus at individual plant level. Scale was another important consideration in my survey designs, for practical reasons associated with conducting each survey, and because species richness is area dependent (Fridley *et al.*, 2007; Lunt, 1990). In all studies I collected community data within a large number of small (0.75m x 0.75m square, or a 0.3m radius circle) plots distributed across the study area. This provided the high resolution needed to observe fine-scale plant responses and interactions between plant groups, and allowed the capture of overall species richness and the comparisons of responses to conditions that vary more widely (Price and Morgan, 2010).

The key variables identified in these surveys were then tested in a controlled field experiment that measured responses by seedlings to treatments, and to changes in structural and resource availability variables. The latter provided a deeper understanding of the underlying mechanisms explaining the responses by seedlings to the treatments. To conclude, I developed a conceptual model, based on our research results and those of others, to represent the main threats to native forbs in temperate grasslands and appropriate management actions for practitioners.

Logistics and limited time-frames allowed for PhDs have prevented me from conducting my research over a wider geographical area and I acknowledge that conducting all my research in one location limits the ability to generalize based solely on my results. This is overcome to some extent by relating results from each of my studies with those obtained in other locations by other researchers, especially as many of the species I studied are widely occurring and also occur in other studied locations. A benefit of conducting three types of study in the same location is that comparisons and consideration of the combined results can be made without having to allow for differences in potentially confounding factors such as geology, weather, landscape species pool and management history.

Research site

The research for this PhD was conducted entirely within temperate grassland within 70 ha of grassy woodland in the Kama Nature reserve, ACT, in south-eastern Australia (35.270562^{o} S, 149.026425^{o} E). The study area was an undulating south west facing (120^{o} < aspect angle < 326^{o}, average = 240^{o}) slope (0.6^{o} < slope < 9.7^{o}, average = 4.0^{o}) and extends from 560 m to 621 m above sea level with a median annual rainfall of 650 mm. Soils are of volcanic origin with areas of sediments, representing a variety of soil types including red kurosols and rocky red kandosols on the higher slopes and grey, red and brown chromosols lower in the landscape (King 1996, terminology converted according to DMR 2002). The soils naturally have low fertility except where super-phosphate was applied in conjunction with the sowing of oats (*Avena sativa*) and subterranean clover (*Trifolium subterraneum*) from as early as 1947 (ACT Government unpublished). The site was declared a nature reserve in 2010 after previously being managed as a pastoral lease, with sheep grazing from the 1920s (ACT Government unpublished) and cattle grazing from 1985-2005. The ground-layer vegetation ranged from highly modified communities dominated by exotic species to long-undisturbed areas dominated by native species (Figure 1.2). There is on-going low intensity kangaroo and rabbit grazing, managed by culling. Fire affected the lower part of the site in 2003, and areas dominated by invasive exotic St John's Wort (*Hypericum perforatum*) were boom-sprayed with a non-residual selective broadleaf herbicide (fluroxypyr) in August 2011 and 2012.

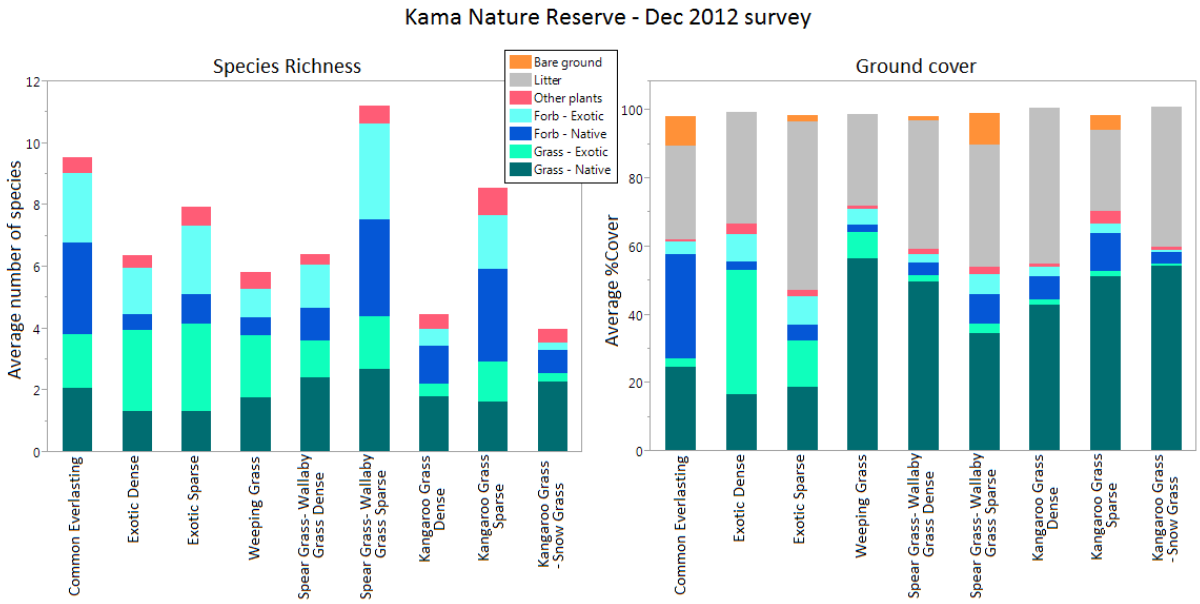

Figure 1.2. The ground layer of the study area (Kama Nature Reserve) divided into nine management units identified during the survey, based on species composition and biomass density.

Thesis aim and research questions

The overarching aim of my research was to contribute to our current understanding of grassland diversity, with a focus on finding better ways to restore and manage self-sustaining populations, and diversity, of native forb species in temperate grasslands. Understanding the different processes affecting species membership in communities is valuable to ecology as well as management (HilleRisLambers *et al.*, 2012). In this thesis I investigate the broader issue of grassland diversity while focusing on native forb habitat requirements and threats, in order to provide information that will help practitioners restore and manage temperate grassland in the future. My high-level research questions were:

1. What are the main factors threatening native plant diversity?
2. What are the main habitat requirements of native forbs?
3. How does competition between plant groups, dominant grasses, native and exotic forbs, influence plant diversity?

4. How should we manage and restore native forb diversity in temperate grassy ecosystems?

Methodological approach

I conducted three quantitative empirical studies to answer the first three research questions and concluded with a synthesis of the results of my empirical studies and existing research. Methods used in each part of the thesis are described in more detail within their respective chapters, but I provide a brief overview here:

For **Chapter Two** my goal was to identify the environmental conditions needed in a suitable restoration site, where native forbs are likely to persist, even with possible competition from exotic forbs. The results of this study may increase our understanding of how exotic species establish themselves in modified grasslands. I was also interested to test whether differences in exotic and native performance are related primarily with origin or are they simply due to differences in longevity (as suggested by several researchers, as most exotic forbs are annuals). I conducted a survey of the ground-layer vegetation and soils in 192 random locations within the study area, stratified by nine different vegetation types, and compared native and exotic forb responses and occupancy likelihood over a broad range of environmental conditions. I analysed soil chemistry and physical properties, and I obtained topographic information from a digital model based on GPS coordinates. I used Bayesian fourth corner analysis in R to predict responses of native and exotic, annual and perennial forbs to eight environmental variables representing landscape, soil, and vegetation biomass.

For **Chapter Three** I was interested in determining the fine-scale variables associated with the presence of a native forb in modified grasslands and whether their influence affected the regeneration niche primarily, or both the regeneration niche and competition for survival. To complement previous research that revealed the importance of physical gap structure for forb habitat quality, I focused on modified grasslands. Forb habitat quality in modified grasslands is typically reduced by an evening out of the physical structure, changed disturbance regimes, and the establishment of exotic forbs and grasses. Knowledge of the influential components in modified grasslands may improve our ability to select appropriate forb restoration sites

and to manage grasslands in ways that maintain forb diversity. I conducted a case-control study of the differences between the vegetation and soil surface conditions in the immediate vicinity of a native forb (the case), and two equivalent areas randomly located within five meters where no native forbs exist (the controls). I analysed data from 145 cases and 290 controls, using conditional logistic regression in R to identify the most significant variables and calculate their influence on the odds ratios of native forb presence/absence.

For **Chapter Four** I conducted a field experiment designed to test responses of native and exotic forb seedlings to a fully-crossed design of four treatments that reduced the level of competition from dominant grasses and exotic species and removed biomass. In addition to trialing plant responses to the treatments, as is commonly done to test the effectiveness of treatments, the I also measured a range of structural and resource variables influenced by the treatments to help us understand the mechanisms behind forb responses to the treatments. I identified these variables previously as having a negative influence on native forbs and from the literature I expected forbs to be most susceptible during seedling emergence. I used GLMM statistical analyses in R to measure seedling emergence responses to the treatments, and to identify the structural and resource variables significantly associated with native and exotic forb responses.

Chapter Five is a synthesis of the results from the three empirical studies and existing research. I present a conceptual model representing native forb habitat suitability in relation to gradients of available soil phosphorus and total biomass (grass and litter combined). I suggest some thresholds for these variables and discuss appropriate actions for restoration and management.

Thesis structure

The thesis is in the form of *compilation*, where I answer my research questions through the publication of articles in the peer-reviewed scientific literature.

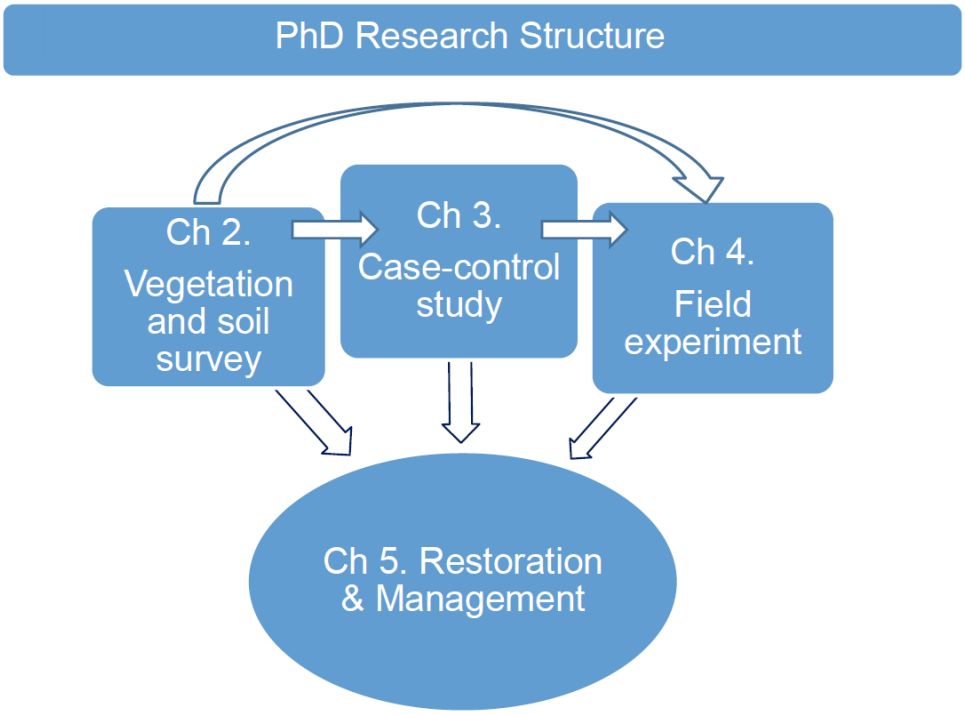

Figure 1.3. Thesis structure. Chapters 2-4 are quantitative empirical studies, each informing the final synthesis in Chapter 5 that provides recommendations for practitioners.

In **Chapter Two** I document a broadly focused survey of the grassland plant communities across my study area. I analysed responses of native and exotic forbs to environmental variables representing landscape, soil, and vegetation biomass. The most influential variables were incorporated into the design of the studies documented in **chapters Three** and **Four**.

In **Chapter Three** I document a case-control study designed to investigate the influence of fine-scale variables on native forb presence/absence. Data collection included the significant variables identified in Chapter Two, as well as variables representing the physical structure of vegetation and soil surface conditions. The most influential variables identified in **chapters Two** and **Three** informed the design of the field experiment documented in **Chapter Four**.

In **Chapter Four** I document a field experiment with treatments designed to test the influence of significant variables from **chapters Two** and **Three** on native forb seedlings, a vulnerable phase for forb species.

Chapter Five concludes the thesis with a synthesis of the results from the three empirical studies in **chapters Two**, **Three** and **Four**, and existing research, in the form of a conceptual model targeting practitioners. I propose some thresholds and discuss appropriate actions for restoration and management of grasslands, depending on soil phosphorus levels and biomass cover.

Research significance

This thesis makes several significant contributions to grassland ecology, restoration and management.

- I highlighted the previously recognized negative effects of biomass and nutrient enrichment on native forb diversity, thereby emphasising the importance of appropriate management of these threats.
- I found that the emergence of native forb seedlings is restricted by a lower threshold of litter mass and are likely to benefit relatively more from litter management than exotic forbs.
- I showed that case-control studies can be an effective approach for native forb research.
- I found few significant differences between the responses of native and exotic forbs, or between annual and perennial forbs, to a range of environmental variables. However, I found that native species were more likely to survive within a restricted range of some variables, soil phosphorus and biomass cover in particular.
- I demonstrated that the differences between the responses of native and exotic forb species to biomass and soil fertility are associated with evolutionary adaptations to the conditions in their region of origin, not differences in the proportion of annuals vs perennials in the group.
- I found that restoration of native forb species cannot rely on sufficient natural recruitment occurring from seed produced by existing populations in and around a potential restoration site.

- I provided a concept model with guidelines to help managers choose appropriate actions for restoration and management depending on soil fertility and biomass conditions.

Chapter Two:

A comparison of native and exotic forb responses to eight variables that influence forb habitat quality in temperate grasslands

This chapter documents a broadly focused random plot survey of the grassland communities occurring at the site. The purpose of this study was to understand how temperate grassland communities respond to environmental variables representing landscape, soil, and vegetation biomass, with a focus on native and exotic forb species. Our analysis compared the responses of annual and perennial native and exotic forbs to look for differences that may be exploited to improve the way we restore and manage native forb diversity.

A version of this chapter has been submitted to Journal of Applied Ecology as:

Johnson D. P., Catford J. A., Driscoll D. A., Blanchard W., Gillen J., & Gibbons P. (Submitted) Plant biomass and available phosphorus key environmental barriers to restoring native forb diversity in a temperate grassland. *Journal of Applied Ecology*.

Abstract

1. Native forb diversity in grasslands has declined following land use change and associated increases in competition from exotic species. Restoring forb diversity in native grassland requires knowledge of the conditions in which native species are able to persist, with or without exotic species. I examined the influence of environmental variables on the composition of annual and perennial exotic and native forb species to identify the conditions in which restoration of native species is likely to be most effective.
2. Using stratified random sampling, I surveyed 192 plots, of 0.75 m x 0.75 m, across 70 ha of temperate grassland in south-eastern Australia. I used Bayesian fourth corner trait analysis to extract trends in the probability of occupancy, and possible environmental range limits, of native and exotic annual and perennial forbs along gradients of aspect, topographic wetness index, grass cover, litter cover, litter depth, soil phosphorus, soil percent clay content, and silt/sand ratio.
3. There was considerable overlap in the occupancy responses of native and exotic forb groups to changes in individual environmental variables. However, exotic species tolerated a wider range of environmental variation than native forb species. Exotic perennial forbs were not limited within the observed range of values for all variables. Exotic annuals were unlikely to occur above 27.1 $mg.kg^{-1}$ of soil phosphorus. Native perennial forbs were unlikely to occur above 11.6 $mg.kg^{-1}$ of soil phosphorus, or in the presence of high litter (> 75%) or grass (> 84%) cover. Native annual forbs were unlikely to occur: outside northern and westerly aspects; where the topographic wetness index or % clay was below average; or where litter cover, litter depth and grass cover were above average.
4. *Synthesis and applications.* Broad overlaps in occupancy responses highlight the difficulty in managing grasslands to favour native forbs over exotic forbs. Management of soil phosphorus and biomass levels should provide native forbs with the conditions in which they can survive, with or without exotic forbs. However, exotic forbs may compete with native forbs, in which case restoration of native forbs may require the removal of those exotic species. In nutrient

enriched soils, exotic forbs are more likely to drive native species decline through increased competition.

Introduction

Natural grasslands are threatened by agriculture, urbanisation, and altered disturbance regimes (McDougall and Morgan, 2005; McIntyre, 2011; Kiehl *et al.*, 2006; Öster *et al.*, 2009; Howe, 1994). This leads to declines in both the areal extent and biodiversity of grasslands, with native forb species particularly affected (Tremont and Mcintyre, 1994; Brandt and Seabloom, 2012; Stevens *et al.*, 2010). Forbs significantly contribute to the floral diversity of native grasslands (Tremont and Mcintyre, 1994; McCain *et al.*, 2010; Pallett *et al.*, 2016) and floral diversity in grasslands is associated with higher levels of protection from soil erosion, water filtration, temporal stability in ecosystem productivity, aesthetic values, and lower levels of exotic plant invasion (Tscharntke *et al.*, 2005; Tilman *et al.*, 2006; Wratten *et al.*, 2012). Native forb diversity also provides food and habitat for other species, including those that perform ecosystem services such as pollination and biological pest control (Schmidt-Entling and Döbeli, 2009; Williams *et al.*, 2015).

Loss of native diversity in grasslands following land use change often coincides with the invasion of exotic plant species (Brandt and Seabloom, 2012; Vilà *et al.*, 2011). This association may be causal, where exotic plants 'drive' native species loss by displacing native species through competitive superiority or by altering local environmental conditions (e.g. availability of light) (Hautier *et al.*, 2009) or disturbance regimes (e.g., fire) (Schlesinger *et al.*, 2013; Scheele *et al.*, 2017; MacDougall and Turkington, 2005). However, the association may be purely correlative, where environmental changes that have prompted the loss of native plants allow exotic species to replace (cf. displace) native species, with exotic species being 'passengers' of the change (MacDougall and Turkington, 2005; Catford *et al.*, 2011).

Native and exotic species—having evolved separately in different parts of the world—may be adapted to, or tolerant of, different environmental conditions (Daehler,

2003; Seabloom *et al.*, 2015; Mod *et al.*, 2016). Where natives and exotics are adapted to, or tolerant of, similar environmental conditions, management designed to increase native forbs could also facilitate exotic species, possibly inadvertently increasing competition (Faithfull *et al.*, 2012; Catford *et al.*, 2012). The success of restoration in grasslands would improve if managers were aware of potential differences in responses by native and exotic forbs to key environmental variables (Prober and Wiehl, 2011; Tognetti and Chaneton, 2015; Driscoll and Scheiner, 2017).

Research comparing native and exotic species often conflates the varying responses of annual and perennial species (Catford *et al.*, 2014; Morgan *et al.*, 2016). That is, the longevity of forbs (annual vs perennial) may also influence their responses to environmental conditions (Dorrough and Scroggie, 2008; Huston and Smith, 1987; Ehrlén and van Groenendael, 1998). Therefore, it is unclear if observed differences in the ecological requirements of native vs exotic forb species stem from their origin, or longevity, as most native forb species in Australia are perennials and a large proportion of exotic forbs are annuals (McIntyre *et al.*, 1995; Seabloom *et al.*, 2015). This is a crucial issue because exotic annuals are commonly considered to have a greater impact on native plant diversity than exotic perennials (Prober and Wiehl, 2011; Seabloom *et al.*, 2015).

I examined associations between the occupancy of sites by native and exotic annual and perennial forb species and eight environmental variables related to landscape position (aspect, topographic wetness index), plant biomass (grass cover, litter cover, litter depth), and soil characteristics (phosphorus, percent clay, and silt/sand ratio). The aim of this study was to identify differences in, (i) the occupancy responses of native and exotic forbs to environmental variables known to influence plant diversity, and (ii) the environmental ranges over which they are likely to occur. The aim of this study was to identify differences in, (i) the occupancy responses of native and exotic forbs to environmental variables known to influence plant diversity, and (ii) the environmental ranges over which they are likely to occur. I were interested in the degree to which forb occupancy or range limits may be influenced by evolutionary adaptations associated with species origin (native vs exotic) and longevity (annual vs perennial). I also discuss the extent to which native and exotic forb

occupancy may have been influenced by land-use history, environmental filtering, and/or competitive interactions.

The study site was chosen because it contained enough variation within each variable to allow us to identify plant responses to each variable, but I acknowledge that the applicability of results based on data from a single location may be geographically limited. However, the benefit of analyzing data collected from a single landscape was that I did not need to allow for variation in factors that may vary on a larger scale, such as geology, weather, species pool and management history. I also acknowledge there are limitations in what a single survey can reveal, due to potential differences between the phenology of individual species. However, I believe I captured the majority of species that are present by conducting the survey during the period when the majority of grassland species are visible.

Methods

Study area

To address these aims I conducted a survey of landscape, vegetation and soil characteristics in a temperate grassland located within a grassy woodland of approximately 70 ha on an undulating south west facing (120^o < aspect angle < 326^o, average = 240^o) slope (0.6^o < slope < 9.7^o, average = 4.0^o) in south-eastern Australia (35.270562^o S, 149.026425^o E). The site extends from 560 m to 621 m above sea level with a median annual rainfall of 650 mm. Soils are of volcanic origin with areas of sediments, representing a variety of soil types including red kurosols and rocky red kandosols on the higher slopes and grey, red and brown chromosols lower in the landscape (King 1996, soil types converted according to DMR 2002). The soils naturally have low fertility except where super-phosphate was applied in conjunction with the sowing of oats (*Avena sativa*) and subterranean clover (*Trifolium subterraneum*) from as early as 1947 (ACT Government unpublished). The site was grazed with sheep from the 1920s, there was low-intensity cattle grazing from 1985-2005 and it was declared a nature reserve in 2010 (ACT Government unpublished). The vegetation ranges from highly modified communities dominated by exotic species to long-

undisturbed areas dominated by native species. There is on-going low intensity kangaroo and rabbit grazing. Fire affected the lower part of the site in 2003 and areas dominated by invasive exotic St John's Wort (*Hypericum perforatum*) were boom-sprayed with a non-residual selective broadleaf herbicide (fluroxypyr) in August 2011 and 2012.

Data collection

I stratified our data collection across nine grassland vegetation types in the study area, identified by the dominant plant species and biomass density (visually assessed) (Table S2.1). I randomly selected 192 plots, stratified by these nine vegetation types. Sampling was undertaken in quadrats measuring 0.75 m x 0.75 m. For each quadrat I recorded or calculated the variables listed in Table 2.1. I analysed two cylindrical soil samples of 5 cm x 5 cm per plot. Thirteen plant taxa could not be identified to species and these were aggregated to genus. The environmental variables and forb traits used in the analysis (Table 2.1) were chosen because they have the potential to influence plant occupancy. None of the potential explanatory variables were highly correlated (Table S2.2). All field data collection was undertaken in Nov-Dec 2012 (austral summer).

Table 2.1. Variables measured for each plot.

(a) Plant species	Definition and measurement method
Species name	Botanical names of vascular plants, according to the Australian Plant Name Index (*http://www.anbg.gov.au/apni/index.html*)
%Cover	Visual estimation of the percentage of area covered by each species.
Native forb P/A	1 or 0, representing the presence or absence of each native forb species
(b) Environmental	
Aspect	An index representing aspect calculated as *Cos(A)*Sin(B)*, where A is the aspect angle relative to NW (315°) (which approximates the hottest and driest aspect in southern Australia) (e.g. if aspect is 200° the relative aspect angle A = 360 - 115 = 245°); B is the slope (in degrees) to moderate for steepness. Aspect is zero where flat or 45° or 225°, positive if < 45° or > 225°, and negative if > 45° and < 225° (Wilkinson and Humphreys, 2006; Hutchinson, 1998).
TWI	Topographic Wetness Index derived from a 20m digital elevation model and calculated as catchment area divided by slope (Moeslund *et al.*, 2013). Ranges from negative in high and sloping areas to positive in low flat areas.
%Grass cover	The total *%Cover* of all the grass species within each plot.
%Litter cover	Visual estimation of the percentage of area covered by dead plant material.
Litter depth	Average of three randomly located measurements of the depth (cm) of dead plant material, lying on the ground or standing.
Available P	Available soil phosphorus in $NaHCO_3$ extract from two randomly located 5 cm x 5 cm soil cores (Colwell, 1963), in $mg.kg^{-1}$.
%Clay	Percentage of a soil core volume occupied by particles of clay size obtained using a Malvin Mastersizer 2000 laser diffraction analyser (Di Stefano *et al.*, 2010).
Silt/Sand ratio	Silt/sand volume ratio of soil cores using Malvin Mastersizer 2000 laser diffraction analyser.
(c) Traits	
Origin	Native or exotic. Source: *http://plantnet.rbgsyd.nsw.gov.au*
Longevity	Annual or perennial. Source: *http://plantnet.rbgsyd.nsw.gov.au*

Analysis

I used fourth corner analysis (Legendre *et al.*, 1997) within the "BORAL" (Bayesian Ordination and Regression Analysis, version 1.01) (Hui, 2016) package in R statistical software (R Core Team, 2016) (see Appendix S2.3 for details) to predict the presence of the j^{th} forb species at the i^{th} site as a function of species' origin, longevity, and their interaction, and environmental covariates, using the probit regression equation with linear predictor (LP_{ij}):

(1) $$LP_{ij} = \beta_{0i} + \beta_{1i}.aspect_j + \beta_{2i}.TWI_j + \beta_{3i}.grass_j + \beta_{4i}.litter_j + \beta_{5i}.litterdepth_j + \beta_{6i}.phosphorus_j + \beta_{7i}.clay_j + \beta_{8i}.siltsand_j + \delta_{1i} + \delta_{2j}$$

δ_{1i} and δ_{2j} in equation (1) are latent variables which allow for variation related to vegetation type, which I used to stratify the study area, and other variables not included in the model (Warton *et al.*, 2015). The β coefficients for the intercept terms (β_{0i}) and each environmental covariate (β_{nj}) are divided into origin and longevity levels and their interaction, plus random effects, ε_{nj}:

(2) $$\beta_{0i} = a_0 + a_1.native_i + a_2.perennial_i + a_3.native{:}perennial_i + \varepsilon_{1j}$$

$$\beta_{1i} = b_0 + b_1.native_i + c_2.perennial_i + b_3.native{:}perennial_i + \varepsilon_{2j}$$

- - - -

$$\beta_{8i} = h_0 + h_1.native_i + h_2.perennial_i + h_3.native{:}perennial_i + \varepsilon_{8j}$$

The model provides posterior distributions for model coefficients for individual species (Equation 1) as well as forb groups according to each origin and longevity level (Equation 2) and the interactions between them. I combined coefficients for origin and longevity to obtain coefficients for occupancy for each origin/longevity forb group. This provided forb group response coefficients for each environmental variable while holding all other variables at their mean (Fig. 2.1, Table S2.3). I also transformed the probit coefficients (using the cumulative normal distribution function, *pnorm* in R) to plot occupancy probabilities of each within-group 'average species' over the recorded range of each environmental variable (Fig. 2.2). I interpreted environmental values for which the upper limit of the credible interval (CI) of a forb group occupancy probability is effectively zero (< 0.001) as being outside the range of species in that forb group for that environmental variable.

Results

A total of 104 plant species were observed across the 192 plots: 41 native forbs, 21 exotic forbs, 18 native grasses, 17 exotic grasses, and seven rushes and sedges (one sedge was exotic). Native grasses were the dominant growth form across the site (38% ± 21% SD cover), followed by native forbs (9% ± 12% SD cover), exotic grasses (8% ± 13% SD cover), exotic forbs (5% ± 7% SD cover), and sedges and rushes (1% ± 4% SD cover). Total perennial and annual species cover were 51% and 10% respectively.

Even though there were more native forb species than exotic forb species across the site (41 vs 21), individual exotic forb species were more widely spread. Exotic annual forbs (16 species) were recorded in 100 plots, exotic perennials (5 species) in 92 plots, native annuals (5 species) in 24 plots, and native perennials (36 species) in 128 plots. Fifty four percent of plots included native and exotic forb species, 15% had natives only, and 19% had exotics only. Table S2.1 contains a summary of the vegetation data, Table S2.2 contains correlations between explanatory variables, Fig. S2.1 contains scatterplots between community response and potential explanatory variables, and Table S2.5 contains a full species list.

Trends in probabilities of forb species occupancy

The median occupancy probability responses and 95% credible intervals (CI) for each forb group to each environmental variable are summarized in Fig. 2.1 and Table S2.3. Forb groups with overlapping CIs for an environmental variable are not significantly different.

The occupancy probability of an exotic annual forb species (i.e. the probability that a typical annual forb species will occupy a plot) increased as aspect approached north-west (i.e. the hottest and driest aspects in southern Australia) and decreased with higher levels of grass cover or available phosphorus (Fig. 2.1). The occupancy probability of native annual forbs declined with increasing litter cover, litter depth and grass cover. The occupancy probability of native perennial forb species declined with increasing litter cover, grass cover, soil clay percentage and available phosphorus, and increased in siltier (cf. sandier) soils. There were different responses by native annual and perennial forbs to litter depth

(Table S2.4a$_{1-3}$). The occupancy of exotic perennial forbs was not significantly associated with any of the variables I examined.

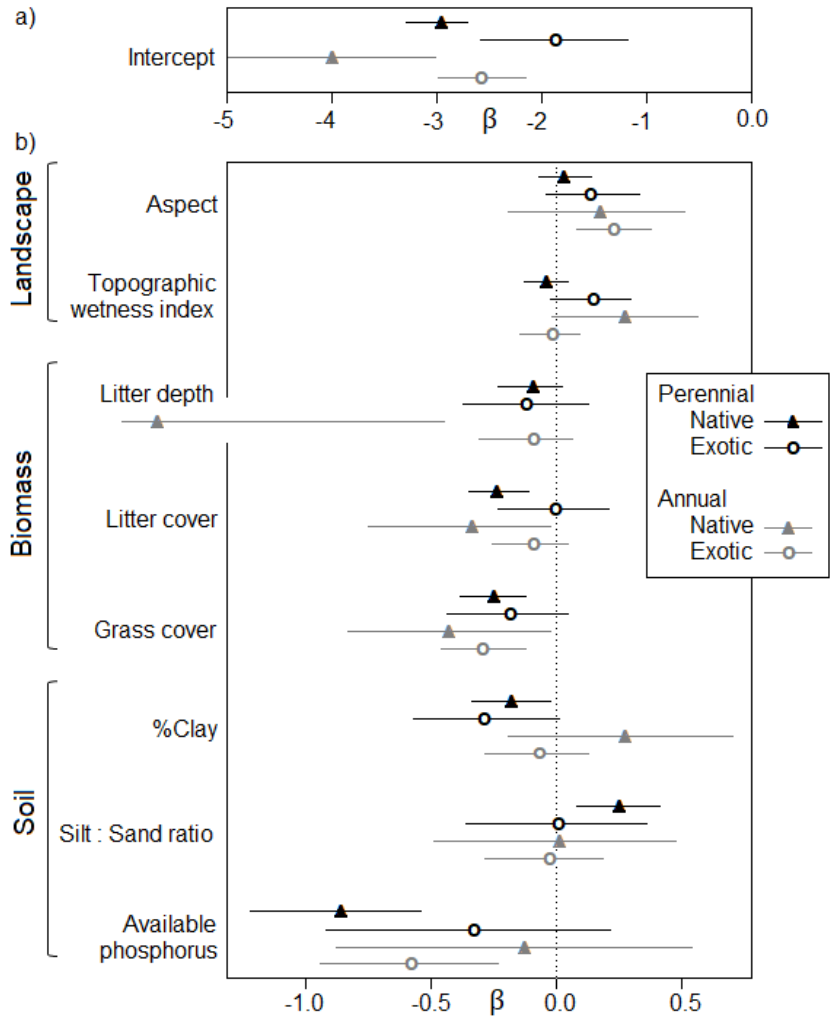

Fig. 2.1. Relative changes in the occupancy responses of the four forb groups (native perennial, exotic perennial, native annual and exotic annual), a) at the intercept (when all environmental variables are at their mean), and b) to increases in each environmental variable. Response coefficients (β) are calculated on the probit scale (median ± 95% credible intervals). Credible intervals that exclude zero can be interpreted as a significant response to the environmental variable. Forb groups with overlapping CIs for an environmental variable are not significantly different.

Environmental range limits

I predicted the probability of occupancy for each forb group (native perennial, exotic perennial, native annual and exotic annual) across the observed range for each environmental variable (Fig. 2.2). I defined a forb group as absent where the upper 95% confidence interval for their predicted probability of occurrence was <0.001.

The environmental range of perennial exotics was not limited within the observed range for any of these variables. Annual exotic forbs were limited to locations where phosphorus was below 27.1 $mg.kg^{-1}$ (Fig. 2.2af). Perennial native forbs were limited to locations where litter cover was <74%, grass cover was <84%, and where phosphorus was <11.6 $mg.kg^{-1}$ (Fig. 2.2q, s, t). Annual native forbs were limited to locations where aspect was >-0.003, TWI >-0.6, %clay >1.4%, litter cover <40%, litter depth <5 cm, and grass cover <48% (Fig. 2.2i-k, y-aa).

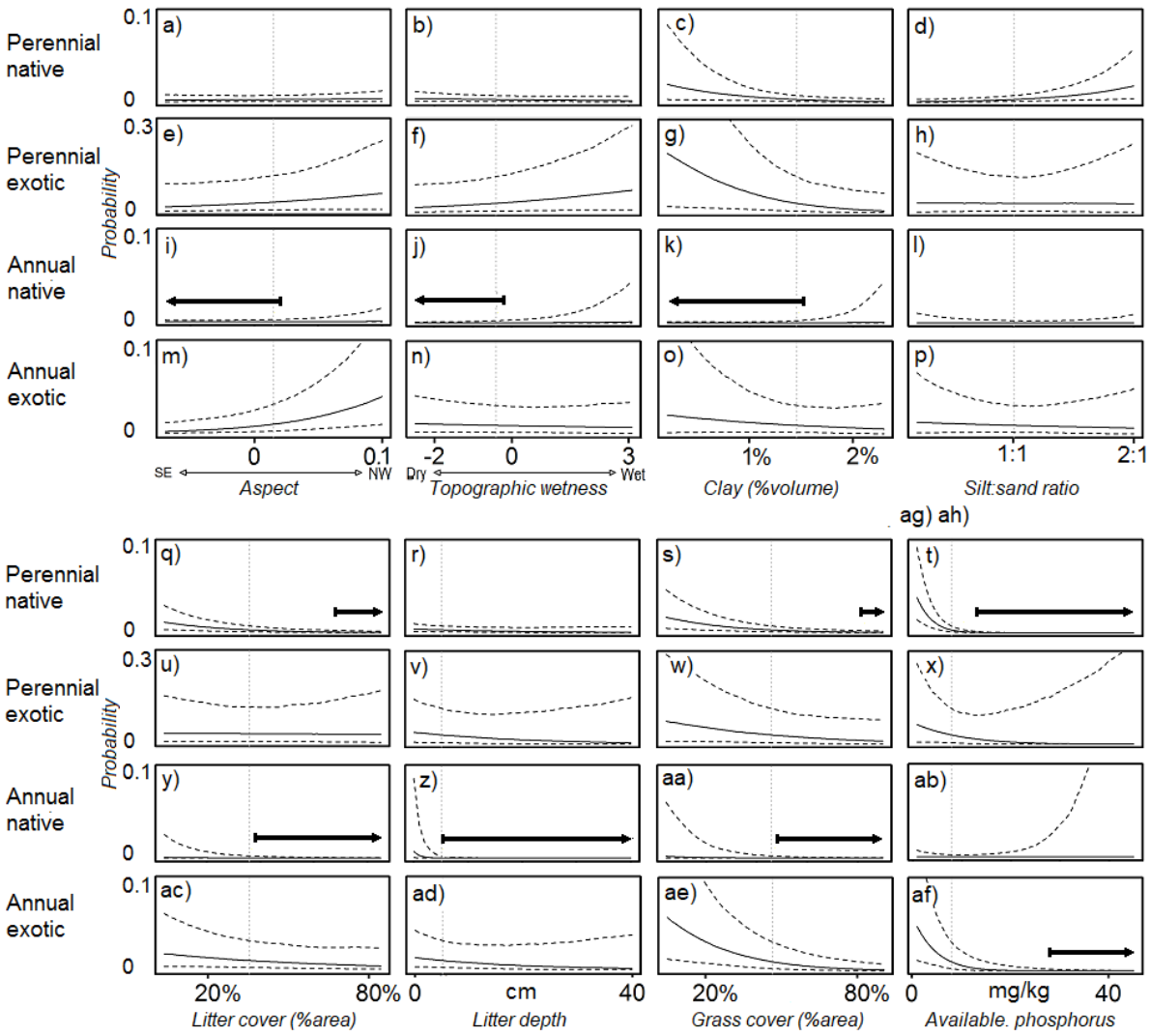

Fig. 2.2. The predicted environmental range of perennial native, annual native and annual exotic forbs. Arrows indicate the range of values for each environmental variable where the forb group is predicted to be absent (i.e., upper 95% confidence interval for the predicted probability of occurrence is <0.001). Predictions for each forb group are given assuming all other environmental variables are held at their mean. The mean observed values for each environmental variable are indicated by vertical dotted lines.

Discussion

I sought to identify environmental conditions in which native forbs may have a greater likelihood of survival, with or without exotic forbs, and therefore if there are conditions in which the restoration or maintenance of native forbs in grasslands may have a higher

likelihood of success. Our analysis of revealed few differences in forb occupancy responses and range limits, of functional groups defined by species origin (native vs exotic) and longevity (annual vs perennial), to eight environmental variables. I found few differences in native and exotic forb occupancy but a high likelihood of differences in the range of soil phosphorus and biomass in which forbs of each group are found. While I may assume relatively uniform land-use history across the site, I can only speculate about the degree to which differences between the distribution of each group are the result of environmental filtering and/or competitive interactions with other species.

The influence of species origin on responses to environmental conditions

Exotic forb species were more likely to occur than native forbs where soil phosphorus and/or biomass levels were high. Trends in forb occupancy along the eight environmental gradients I examined revealed few differences between forb groups, indicating little influence of species origin and longevity. I did find, though, that native species were more likely to occur within a narrower range of environmental conditions than exotic species, especially when just considering annual species. Native species showed restricted distributions along (observed) gradients of grass and litter cover; native perennials were unlikely to occur in phosphorus enriched sites; and native annuals occurred within limited ranges of aspect, topographic wetness and soil clay content. Exotic species were able to occupy all environmental conditions observed across our study site (i.e. upper CI for Pr(occupancy) > 0.001), with the exception of annual exotics, which were unlikely to occur in sites with high soil phosphorus (though they still occurred in plots with higher levels of soil phosphorus than native perennial species).

Exotic forbs were widespread across our study area, occurring in 73% of all sampled plots. However, the processes that lead to the replacement of native forbs by exotic forbs is likely to vary under different environmental conditions. Elevated phosphorus is likely to support high biomass production in exotic forbs, thereby allowing them to 'drive' native species decline by displacing them through light deprivation (MacDougall and Turkington, 2005; Hautier *et al.*, 2009). In exotic-dominated areas with high biomass and

low phosphorus, exotic species may replace native forbs, as 'passengers' of the increases in grass and litter cover that result in declines of native forbs.

Critically, the observed dominance of exotic forbs were not associated with differences in longevity (i.e., annual or perennial). Our results suggest that exotic species have higher overall occupancy because they have broader environmental ranges than native species, possibly due to greater spatial and temporal heterogeneity in environmental conditions during evolution (Kassen, 2002). The high occupancy probability of exotic perennial forbs suggests they are particularly well-suited to Australian conditions, possibly due to adaptations conferring both high growth rate and long life. However, I acknowledge there are other potential sources of differentiation between the occupancy of native and exotic species, such as competitive ability (Huston, 2004), historical land use and propagule availability (Buckley and Catford, 2016), ecological requirements of the individual species present (Vilà and Weiner, 2004), and phylogenetic diversity (Fridley and Sax, 2014).

One key difference between native forbs and exotic forbs was their tolerance to dead and living biomass. Exotic forbs had greater tolerance of dead and living biomass (litter and grass) than native forbs. High levels of litter cover (>40% for annuals, >74% for perennials) and litter depth (>5 cm, annuals only) restricted native species only, likely through reduced light availability and physical obstruction of emerging seedlings (Letts *et al.*, 2015; Johnson *et al.*, 2018). Similarly, native forbs were effectively absent under higher levels of grass cover (>48% for annuals, >84% for perennials) while exotic forbs may be present. Grass cover in our survey represents living grasses only, so the mechanism of restriction may be competition for limited nutrients, moisture, and/or light, although in productive grasslands light is often the limiting resource (Borer *et al.*, 2014; Hautier *et al.*, 2009). Native forbs from grasslands in Australia evolved with less grass and litter cover than European species—due to relatively greater productivity in European grasslands, and to a greater relative frequency of fire in Australia (Groves *et al.*, 2003)–and are less likely than European species to tolerate low light conditions.

Among perennial species, exotic forbs were able to occur in higher levels of soil phosphorus than native forbs. Increased soil phosphorus is associated with the overall

decline of plant species richness (Ceulemans *et al.*, 2013), and is known to disproportionally affect Australian native grassland species more than European species (Seabloom *et al.*, 2015; Dorrough and Scroggie, 2008; Driscoll *et al.*, 2018). The increasing likelihood of exotic perennial forbs in areas with greater phosphorus supports the observation by Dawson et al. (2012) that globally widespread species are better competitors in high resource availability. Our results suggest that native perennial forbs are able to survive with exotic plant species below 11.6 $mg.kg^{-1}$ phosphorus (assuming < 74% litter and < 84% grass cover), but at higher phosphorus levels they are vulnerable to elimination through competition for light by larger and faster growing species, which may include grasses as well as exotic forbs (Hautier *et al.*, 2009).

The influence of longevity

Our results suggest that higher occupancy among exotic species is because of evolutionary adaptations to conditions in their region of origin, not the greater representation of annuals among exotic forb species. This finding is consistent with other studies that found little influence of longevity on forb occupancy or community dynamics (Scharfy *et al.*, 2011; Dorrough and Scroggie, 2008; Meiners, 2007). While the overall impact of exotic annuals on native diversity may be greater than exotic perennials (Prober and Wiehl, 2011; Seabloom *et al.*, 2015), I found that the probability that an individual species will occur is influenced more by its origin than longevity. Nevertheless, annual forbs were more likely to occur within a restricted range of the studied environmental variables than perennial forbs.

Range restrictions among annual species are probably a consequence of them investing more in traits associated with reproduction (e.g. seed production, rapid maturity) and less in traits associated with survival (e.g. energy storage, defence), thus being less able to survive long periods of hardship, than perennial species (Wenk and Falster, 2015). Short-lived species rely more on disturbance for recruitment than long-lived species which can survive long periods without disturbance (Hobbs and Huenneke, 1992). Frequency of, or time since, disturbance may also explain the mismatch between our results and those of Prober and Wiehl (2011) who suggest that annual species respond more positively to nutrients than perennial species. Indeed, other research suggests that longevity-related differences in occupancy are more likely associated with temporal variables (year, season,

time since disturbance) (Brandt and Seabloom, 2011). In the absence of disturbance, short-lived species are gradually succeeded by perennial species (Huston and Smith, 1987).

Implications for management

Broad overlap in responses by native and exotic forbs to the environmental variables measured in this study highlight the difficulty in managing grasslands to favour native forbs over exotic forbs (Huston, 2004; Driscoll and Scheiner, 2017). However, our results suggest that knowing the maximum levels of biomass and soil phosphorus in which native species are likely to occur may improve our ability to restore and maintain conditions suitable for native forb diversity, perennial and annual. Daehler (2003) proposed a strategy for grassland management based around: protecting intact systems, choosing suitable restoration sites, and managing fertility and disturbance regimes to favour native species. However, actions that reduce or avoid high soil fertility (Ceulemans *et al.*, 2011; Prober *et al.*, 2005) or reduce biomass (Prober *et al.*, 2007; MacDougall and Turkington, 2007) may inadvertently increase the likelihood of exotic forbs outcompeting native species (Daehler, 2003), depending on the species present.

Native species will not recover from biomass-induced decline until 'native-friendly' biomass conditions are restored, for example by re-introducing historic burning regimes or applying substitute treatments that achieve similar outcomes (Bauer, 2012; MacDougall and Turkington, 2005). Recovery may also depend on exotic species removal if they are restricting the recovery of native species, the method of removal (if required), and propagule abundance of native and exotic species (Kettenring and Adams, 2011; Johnson *et al.*, 2018). In phosphorus enriched areas, exotic species may have displaced native species through competitive superiority in abundant resource conditions (Seabloom *et al.*, 2015; Hautier *et al.*, 2009). In this case, the removal of exotic forbs will not necessarily lead to an increase in native forb diversity, even if biomass levels are low, as exotic forbs may re-invade, or other grassland plants (e.g. exotic grasses) may continue to compete with native forbs (Kettenring and Adams, 2011; Bauer, 2012). Native forbs are more likely to benefit from biomass reduction in areas with low soil fertility than areas with high fertility.

Unfortunately, reversing soil nutrient enrichment (by phosphorus in particular) is currently not feasible in the short term (Ceulemans *et al.*, 2013).

Our results build on previous evidence that native forbs are more restricted by biomass than exotic species (Johnson *et al.*, 2018), and that site selection for grassland restoration should consider current and future soil fertility, litter cover, grass cover, and potential sources of incoming exotic propagules (Hobbs and Huenneke, 1992; Daehler, 2003). I suggest that 'native friendly' locations (with low biomass and phosphorus levels) with relatively low richness and/or cover of native forbs have the greatest potential for restoration to achieve gains in native forb richness and/or population size, depending on the level of competition from exotic species. On-going management should periodically remove excess biomass if required (Morgan, 2015). However, this approach will not exclude exotic forbs, so ongoing monitoring for arrivals and population changes of exotic forbs will be necessary, particularly following disturbance or unusually wet weather (Huston, 2004). Minimising the chances of accidentally introducing new undesirable exotic species is also important (Daehler, 2003).

In practice, the on-going spread of exotic species and environmental changes (e.g. climate change) may make the restoration of native forb diversity difficult to achieve (Hobbs *et al.*, 2006). The success of restoration actions may be dependent on other factors (e.g. soil texture, climate) (Pennington *et al.*, 2017). Even small niche differences may facilitate co-existence of species by overcoming the effects of differences in competitive ability (Staples *et al.*, 2016), although this may take a long time (McIntyre *et al.*, 2017).

Author's contributions

DPJ, PG and JG conceived and conducted the survey; DPJ and JG designed and conducted the soil analyses; DPJ developed the concept for the paper in collaboration with JAC, DAD and PG, based on a statistical approach conceived by WB; DPJ analysed the data in consultation with WB; DPJ wrote the paper with feedback and editorial advice from JAC, DAD and PG, reviewed by JG and WB.

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Data accessibility

All data and code used in the analysis will be made available through the Dryad Digital Repository.

Appendix S2.1

Table S2.1. Nine vegetation community types, and summary data for characteristic information collected.

Community	Typical dominant species		Species richness (all species)	Forb richness	Elevation (m)	Aspect NW index	Topographic wetness index (TWI)	% Grass cover	% Bare Ground	% Litter cover	Litter Depth (cm)	Bulk Density (gm/cc)	pH	EC (mS/cm)	% Clay	% Silt / % Sand	Available P (mg/kg)	C/N Ratio
All combined		MIN	1	0	560	-0.07	-2.47	4	0	4	0	1.3	4.7	21	0.2	0.3	0.8	9.4
n=192		MAX	20	14	621	0.10	3.05	90	35	85	40	3.0	6.8	301	2.3	3.2	44.3	19.2
		AVG	7.2	3.3	588	0.01	-0.41	46	3	36	5	2.2	5.6	87	1.5	1.0	7.8	11.6
		SD	3.6	2.7	15	0.03	1.08	19	7	17	6	0.3	0.4	59	0.4	0.4	6.6	1.3
Chrysocephalum	*C. apiculatum*	MIN	3	1	563	-0.01	-2.07	4	0	5	0	2.1	5.4	25	0.4	0.4	0.8	9.4
n=24		MAX	15	9	602	0.08	0.85	52	30	55	5	3.0	6.7	137	2.2	1.2	8.4	18.3
		AVG	9.4	5.2	581	0.03	-0.46	26	9	28	2	2.5	5.8	43	1.5	0.8	4.0	11.6
		SD	2.8	1.9	10	0.02	0.89	10	10	13	1	0.2	0.3	22	0.4	0.2	1.7	2.1
Exotic Dense	*Avena sativa*	MIN	1	0	564	-0.05	-2.08	18	0	15	2	1.3	4.7	56	0.2	0.3	4.9	9.8
n=22	*Hypericum perforatum*	MAX	15	8	621	0.07	2.76	80	5	55	12	2.7	6.8	301	2.2	3.2	44.3	13.2
	Phalaris spp	AVG	6.5	2.1	588	0.03	-0.12	54	0	31	6	1.9	5.7	128	1.2	1.0	15.6	11.6
	Trifolium spp	SD	3.1	1.8	18	0.03	1.28	17	1	11	2	0.3	0.6	62	0.4	0.6	11.2	0.9
	Bromus spp																	
Exotic Sparse	*Avena sativa*	MIN	2	0	567	-0.05	-2.31	9	0	20	1	1.6	4.7	21	0.8	0.6	3.8	9.6
n=27	*Lolium perenne*	MAX	18	11	621	0.08	2.41	65	20	85	5	2.7	6.1	229	2.1	1.7	29.5	13.7
	Vulpia spp	AVG	8.0	3.3	595	0.01	-0.33	32	2	51	2	2.1	5.5	85	1.4	0.9	9.7	11.5
	Trifolium spp	SD	3.5	2.4	18	0.03	1.08	15	6	15	1	0.3	0.4	55	0.3	0.3	6.6	1.1
	Bromus spp																	
Microlaena	*Microlaena stipoides*	MIN	1	0	568	-0.06	-1.82	36	0	10	2	1.3	5.2	60	0.3	0.3	6.8	10.2
n=20		MAX	9	4	618	0.05	1.89	90	0	55	40	2.2	6.4	243	2.2	2.0	36.8	13.9
		AVG	5.8	1.6	591	0.00	-0.46	65	0	26	8	1.7	5.9	111	1.2	1.0	12.6	11.9
		SD	2.4	1.2	15	0.03	0.87	13	0	11	11	0.2	0.3	50	0.4	0.4	7.1	0.9
Stipa-Dan Dense	*Austrostipa spp*	MIN	1	0	564	-0.05	-2.47	10	0	15	1	1.5	5.1	32	0.9	0.5	2.7	9.5
n=21	*Rytidosperma spp*	MAX	12	7	621	0.08	1.66	75	15	65	30	2.7	6.2	142	1.9	1.4	11.6	14.8
		AVG	6.2	2.5	594	0.02	-1.01	49	1	40	6	2.2	5.4	60	1.4	0.9	7.1	11.8
		SD	3.2	2.3	15	0.03	0.89	15	3	13	7	0.3	0.3	30	0.2	0.2	2.6	1.4
Stipa-Dan Sparse	*Austrostipa spp*	MIN	5	1	560	0.00	-2.33	10	0	10	0	2.0	5.1	23	0.8	0.5	1.9	10.0
n=22	*Rytidosperma spp*	MAX	20	14	614	0.10	3.05	57	35	70	4	3.0	6.2	103	2.3	1.5	8.4	13.6
		AVG	10.8	6.1	588	0.03	-0.47	35	9	38	1	2.5	5.6	43	1.7	0.9	4.6	11.5
		SD	3.5	3.1	15	0.02	1.26	12	12	17	1	0.2	0.3	18	0.4	0.2	1.9	1.0
Themeda Dense	*Themeda triandra*	MIN	2	0	570	-0.06	-1.67	12	0	8	4	1.8	4.8	32	1.0	0.7	2.5	10.3
n=17		MAX	9	6	602	0.07	1.39	80	0	85	16	2.6	6.0	249	2.0	1.9	12.3	12.2
		AVG	4.4	1.9	591	-0.01	-0.19	46	0	43	9	2.2	5.3	139	1.6	1.3	6.6	11.2
		SD	2.2	1.7	7	0.04	1.05	20	0	22	4	0.2	0.3	65	0.3	0.4	3.1	0.6
Themeda Sparse	*Themeda triandra*	MIN	5	2	564	-0.04	-2.29	35	0	4	1	1.9	5.0	27	1.0	0.6	1.5	10.2
n=21		MAX	13	11	599	0.05	1.41	76	25	45	30	2.7	6.0	169	2.2	2.0	9.0	19.2
		AVG	8.4	4.9	583	0.01	-0.45	56	3	24	5	2.4	5.6	69	1.6	1.2	4.1	12.0
		SD	2.0	2.0	11	0.03	1.06	11	7	12	7	0.2	0.2	36	0.3	0.3	2.2	1.9
Themeda-Poa	*Themeda triandra*	MIN	2	0	573	-0.07	-1.94	31	0	20	4	2.0	5.2	50	1.1	0.6	2.1	10.3
n=18	*Poa sieberiana*	MAX	6	3	592	0.06	1.47	75	0	70	18	2.5	6.0	295	1.8	2.0	8.9	12.2
		AVG	3.8	1.0	580	-0.02	-0.14	56	0	40	11	2.2	5.7	131	1.5	1.3	5.1	11.1
		SD	1.3	0.9	5	0.04	0.96	12	0	13	4	0.1	0.2	65	0.2	0.4	2.1	0.5

Appendix S2.2

Table S2.2. Pearson correlation matrix for the variables used in the analysis. AspNW = Aspect NW index; TWI = Topographic wetness index; Litter = %Cover litter; LitDepth = Litter depth; PcGrass = %Cover of grasses; pctClay = % of soil volume made up of clay-sized particles; AvailP = Available soil phosphorus; Silt_Sand = ratio of silt-sized particles to sand-sized particles, both as % of soil volume.

	AspNW	TWI	Litter	LitDepth	PcGrass	pctCLAY	AvailP	Silt_Sand
AspNW	1.00	0.15	-0.18	-0.19	-0.11	-0.14	-0.04	-0.44
TWI	0.15	1.00	-0.04	0.05	0.02	-0.02	-0.01	0.10
Litter	-0.18	-0.04	1.00	-0.01	-0.53	0.04	0.03	0.09
LitDepth	-0.19	0.05	-0.01	1.00	0.29	-0.08	0.03	0.20
PcGrass	-0.11	0.02	-0.53	0.29	1.00	-0.21	0.21	0.03
pctCLAY	-0.14	-0.02	0.04	-0.08	-0.21	1.00	-0.47	0.55
AvailP	-0.04	-0.01	0.03	0.03	0.21	-0.47	1.00	-0.19
Silt_Sand	-0.44	0.10	0.09	0.20	0.03	0.55	-0.19	1.00

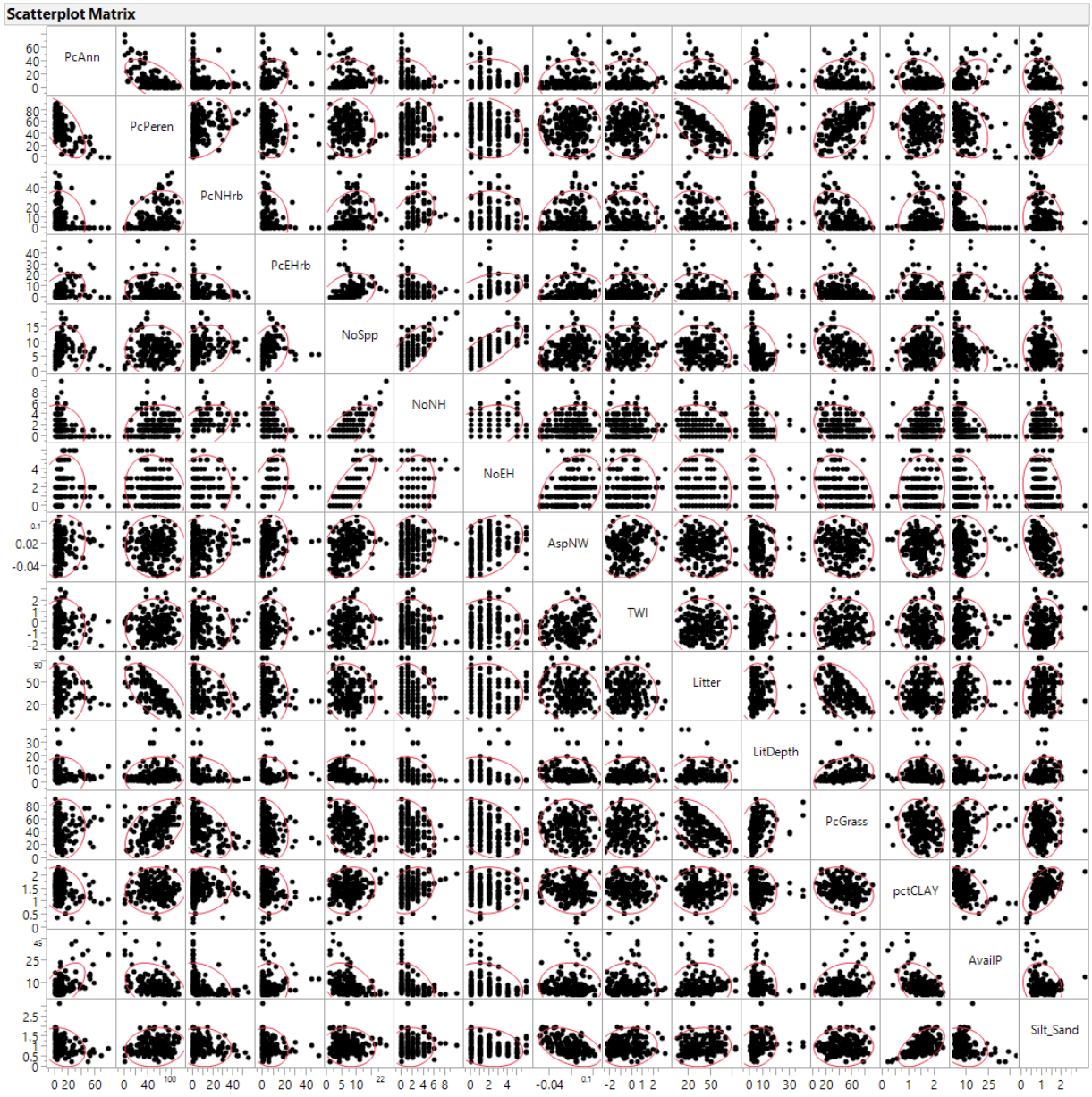

Fig. S2.1. Scatterplots for vegetation community and measured variables. PcAnn = %Cover of annuals; PcPeren = %Cover of perennials; PcNHrb = %Cover of native forbs; PcEHrb = %Cover of exotic forbs; NoSpp = Species richness (all species); NoNH = Species richness (native forbs); NoEH = Species richness (exotic forbs); AspNW = Aspect NW; TWI = Topographic wetness index; Litter = %Cover litter; LitDepth = Litter depth; PcGrass = %Cover of grasses; pctClay = % of soil volume made up of clay-sized particles; AvailP = Available soil phosphorus; Silt_Sand = ratio of silt-sized particles,

both as % of soil volume. Elongated density ellipses indicate a level of correlation between variable pairs.

Appendix S2.3

Details of Model Execution and Checking

Prior to analysis I rescaled the environmental covariates to have mean zero and variance one, to enhance MCMC convergence stability (Dorrough and Scroggie, 2008). This also meant that the longevity-origin response coefficients for each environmental variable were comparable, and coefficients with 95% credible intervals (CIs) not including zero could be identified as being significant.

I ran the analysis with BORAL's deliberately un-informative default prior distribution parameters (Hui, 2016). BORAL uses the prior distribution parameters in combination with the probit regression to estimate the posterior distributions for each variable using Bayesian Markov chain Monte Carlo (MCMC) sampling from a single chain. I specified the inclusion of two latent variables to account for variation not related to the covariates in the model (Hui, 2016). I specified 100,000 iterations in total for the MCMC chain from which the first 10,000 were ignored as burn-in, to avoid distortion before convergence had stabilised. Every 30^{th} iteration was retained from the 90000 remaining after burnin, to minimize the effects of auto-correlation in the posterior samples. Experimenting with different prior distribution parameters did not change the results of the analysis. I visually checked plots of a subset of the chain coefficients, and auto-correlation function plots, to check MCMC convergence. I also used Geweke diagnostics (Cowles and Carlin, 1996) to confirm that the chain has a high probability of convergence.

Interpreting Model Results

The Boral MCMC output includes posterior distributions for model coefficients for each individual species response to every variable, as well as by trait level. Trait level coefficients are additive, with the coefficients for exotic-annuals as the baseline. Coefficients for the other forb groups require calculation of the baseline plus the coefficients representing the additional effect of each level for that forb group (Table S2.5).

The coefficients for exotic annual responses (Table S2.4a) to each variable is the (baseline) coefficient for exotic-annuals responses (Table S2.5, a_0). The coefficients for native annual responses (Table S2.4b) equals the baseline coefficient plus the coefficient for the native level of the origin trait (Table S2.5, $a_0 + a_1$). The coefficients for exotic perennial responses (Table S2.2.4c) the baseline coefficient plus the coefficient for the perennial level of the longevity trait (Table S2.5, $a_0 + a_2$). The coefficients for native perennial responses (Table S2.4d) equals the baseline coefficient plus the coefficients for the native level of the origin trait, the perennial level of the longevity trait, and the interaction of the native level of the origin trait and perennial level of the longevity trait (Table S2.5, $a_0 + a_1 + a_2 + a_3$).

Table S2.3. Modelled forb occupancy responses of each forb group on the probit scale. The intercept row (grey background) represents the occupancy of each origin-longevity forb group–(a) exotic annual, (b) native annual, (c) exotic perennial, and (d) native perennial– when all environmental variables are at their mean value. The other rows represent posterior coefficient medians and the extremes of 95% credible intervals (2.5% - 97.5%) for each origin-longevity forb group occupancy response to changes in aspect NW, topographical wetness index, %litter cover, litter depth, %grass cover, %clay, available phosphorus, and silt/sand ratio. Responses with credible intervals excluding zero (in bold) are considered significant.

	a) Exotic Annual (n=16)			b) Native Annual (n=5)			c) Exotic Perennial (n=5)			d) Native Perennial (n=36)		
Variable	Med	2.5%	97.5%	Med	2.5%	97.5%	Med	2.5%	97.5%	Med	2.5%	97.5%
Intercept	**-2.57**	**-3.04**	**-2.14**	**-4.09**	**-5.31**	**-3.04**	**-1.87**	**-2.60**	**-1.17**	**-2.97**	**-3.32**	**-2.67**
AspNW	**0.22**	**0.07**	**0.37**	0.17	-0.20	0.51	0.13	-0.07	0.34	0.03	-0.08	0.14
TWI	-0.03	-0.16	0.09	0.25	-0.03	0.56	0.13	-0.06	0.32	-0.05	-0.15	0.05
%Litter	-0.10	-0.27	0.05	-0.36	-0.76	0.00	-0.01	-0.24	0.22	**-0.24**	**-0.36**	**-0.11**
LitDepth	-0.11	-0.33	0.07	**-1.61**	**-3.19**	**-0.44**	-0.13	-0.40	0.12	-0.10	-0.25	0.03
%Grass	**-0.30**	**-0.49**	**-0.13**	**-0.44**	**-0.87**	**-0.01**	-0.19	-0.48	0.06	**-0.26**	**-0.40**	**-0.12**
%Clay	-0.08	-0.29	0.12	0.25	-0.21	0.72	-0.29	-0.60	0.01	**-0.19**	**-0.35**	**-0.02**
AvailP	**-0.58**	**-0.97**	**-0.22**	-0.13	-0.84	0.57	-0.34	-0.92	0.25	**-0.87**	**-1.22**	**-0.57**
Silt/Sand	-0.05	-0.30	0.20	-0.01	-0.52	0.48	-0.01	-0.38	0.36	**0.24**	**0.06**	**0.41**

Table S2.4. Posterior coefficient medians and 95% credible intervals for the additive trait-level coefficients for combination and interaction, for each environmental variable. Exotic-annual (a_0) is the baseline, (a_1) shows the effect of changing origin to native, (a_2) shows the effect of changing longevity to perennial, (a_3) shows the interaction effect of changing both origin and longevity. Responses with credible intervals excluding zero (in bold) are considered significant.

	a_0 (Exotic-Annual)			a_1 (+ Native)			a_2 (+ Perennial)			a_3 (+ Native + Peren)		
Variable	Med	2.5%	97.5%	Med	2.5%	97.5%	Med	2.5%	97.5%	Med	2.5%	97.5%
Intercept	**-2.59**	**-3.06**	**-2.14**	**-1.54**	**-2.82**	**-0.39**	0.69	-0.16	1.55	0.35	-1.01	1.68
AspNW	**0.22**	**0.07**	**0.38**	-0.05	-0.46	0.33	-0.10	-0.34	0.17	-0.04	-0.46	0.44
TWI	-0.03	-0.17	0.09	0.29	-0.04	0.62	0.17	-0.04	0.39	**-0.46**	**-0.80**	**-0.08**
%Litter	-0.10	-0.27	0.05	-0.25	-0.70	0.13	0.09	-0.18	0.36	0.05	-0.45	0.55
LitDepth	-0.10	-0.32	0.06	**-1.51**	**-3.09**	**-0.31**	-0.02	-0.34	0.34	**1.44**	**0.32**	**2.64**
%Grass	**-0.30**	**-0.47**	**-0.13**	-0.14	-0.57	0.35	0.11	-0.21	0.41	0.08	-0.48	0.62
%Clay	-0.08	-0.30	0.13	0.34	-0.17	0.81	-0.21	-0.58	0.15	-0.23	-0.82	0.40
AvailP	**-0.58**	**-0.96**	**-0.23**	0.44	-0.42	1.21	0.24	-0.45	0.94	-0.95	-1.92	0.07
Silt/Sand	-0.04	-0.30	0.18	0.04	-0.49	0.60	0.05	-0.39	0.50	0.21	-0.48	0.86

Appendix S2.4

Table S2.5. Species list of recorded plants, including longevity, the number of plots and average cover.

Species by plant type	Family	Longevity	No Plots	Avg %Cover (if present)
Grasses - Native				
Austrostipa bigeniculata	Poaceae	Perennial	35	20
Austrostipa scabra	Poaceae	Perennial	46	15
Bothriochloa macra	Poaceae	Perennial	2	3
Dichelachne crinita	Poaceae	Perennial	1	10
Dichelachne sieberiana	Poaceae	Perennial	3	5
Elymus scaber	Poaceae	Perennial	32	5
Eragrostis brownii	Poaceae	Perennial	1	1
Microleana stipoides	Poaceae	Perennial	35	36
Panicum effusum	Poaceae	Perennial	3	1
Poa sieberiana	Poaceae	Perennial	24	17
Rytidosperma auriculatum	Poaceae	Perennial	1	25
Rytidosperma broadleaf	Poaceae	Perennial	1	5
Rytidosperma laeve	Poaceae	Perennial	1	1
Rytidosperma setaceum	Poaceae	Perennial	5	26
Rytidosperma short	Poaceae	Perennial	10	13
Rytidosperma spp	Poaceae	Perennial	35	14
Rytidosperma tall	Poaceae	Perennial	20	7
Themeda australis	Poaceae	Perennial	111	28
Grasses - Exotic				
Aira caryophyllea	Poaceae	Annual	13	1
Aira cupaniana	Poaceae	Annual	24	1
Aira elegantissima	Poaceae	Annual	22	1
Aira spp	Poaceae	Annual	1	1
Avena sativa	Poaceae	Annual	20	15
Briza minor	Poaceae	Annual	15	2
Bromus catharticus	Poaceae	Annual	3	53
Bromus diandrus	Poaceae	Annual	19	8
Bromus hordaceous	Poaceae	Annual	61	2
Bromus rubens	Poaceae	Annual	3	9
Cynosurus echinatus	Poaceae	Annual	1	4
Festuca arundinacea	Poaceae	Perennial	2	6
Holcus lanatus	Poaceae	Annual	3	8
Hordeum spp	Poaceae	Annual	3	2
Lolium perenne	Poaceae	Perennial	21	3

Phalaris aquatica	Poaceae	Perennial	10	18
Vulpia spp	Poaceae	Annual	94	4
Forbs - Native				
Acaena ovina	Rosaceae	Perennial	2	1
Asperula conferta	Rubiaceae	Perennial	4	3
Bulbine bulbosa	Asphodelaceae	Perennial	7	1
Cheilanthes austrotenuifolia	Pteridaceae	Perennial	10	3
Cheilanthes sieberi	Pteridaceae	Perennial	1	5
Chrysocephalum apiculatum	Asteraceae	Perennial	39	22
Convolvulus erubescens	Convolvulaceae	Perennial	6	1
Crassula sieberiana	Crassulaceae	Perennial	4	3
Cymbonotus preissianus	Asteraceae	Perennial	1	2
Daucus glochidiatus	Apiaceae	Annual	1	1
Drosera peltata	Droseraceae	Perennial	1	1
Erodium crinitum	Geraniaceae	Annual	1	25
Eryngium ovinum	Apiaceae	Perennial	5	4
Euchiton gymnocephalus	Asteraceae	Perennial	2	1
Euchiton involucratis	Asteraceae	Perennial	4	3
Euchiton sphaericus	Asteraceae	Annual	9	1
Geranium solanderi	Geraniaceae	Perennial	1	5
Glycine clandestina	Fabaceae	Perennial	1	2
Glycine tabacina	Fabaceae	Perennial	17	1
Gonocarpus tetragynus	Haloragaceae	Perennial	7	2
Goodenia hederacea	Goodeniaceae	Perennial	4	1
Goodenia pinnatifida	Goodeniaceae	Perennial	1	5
Haloragis heterophylla	Haloragaceae	Perennial	11	10
Hydrocotyle laxiflora	Apiacaea	Perennial	27	5
Hypericum gramineum	Clusiaceae	Either	6	4
Leptorhynchos squamatus	Asteraceae	Perennial	14	3
Microtis unifolia	Orchidaceae	Perennial	1	1
Oxalis perennans	Oxalidaceae	Perennial	18	1
Pimelea curviflora	Thymelaeaceae	Perennial	1	10
Plantago gaudichaudii	Plantaginaceae	Perennial	1	3
Plantago varia	Plantaginaceae	Perennial	16	6
Rumex brownii	Polygonaceae	Perennial	3	2
Senecio quadridentatus	Asteraceae	Perennial	1	1
Solenogyne dominii	Asteraceae	Perennial	15	2
Tricoryne elatior	Anthericaceae	Perennial	17	1
Triptilodiscus pygmaeus	Asteraceae	Annual	17	2
Velleia paradoxa	Goodeniaceae	Perennial	1	1
Vittadinia cuneata	Asteraceae	Either	3	3

Vittadinia muelleri	Asteraceae	Perennial	19	5
Wahlenbergia communis	Campanulaceae	Perennial	3	1
Wahlenbergia stricta	Campanulaceae	Perennial	1	1
Forbs - Exotic				
Acetosella vulgaris	Polygonaceae	Perennial	24	4
Anagallis arvensis	Primulaceae	Annual	1	1
Centaurium erythraea	Gentianaceae	Annual	17	1
Centaurium lineafolium	Gentianaceae	Annual	2	2
Centaurium tenuiflorum	Gentianaceae	Annual	7	1
Chondrilla juncea	Asteraceae	Perennial	7	2
Conyza sumatrensis	Asteraceae	Annual	12	1
Hypericum perforatum	Clusiaceae	Perennial	36	6
Hypochaeris radicata	Asteraceae	Perennial	43	2
Lactuca serriola	Asteraceae	Annual	1	1
Orobanche minor	Scrophulariaceae	Annual	3	2
Parentucellia latifolia	Scrophulariaceae	Annual	1	1
Petrorhagia nanteuilii	Caryophyllaceae	Annual	44	1
Plantago lanceolata	Plantaginaceae	Annual	14	7
Sonchus oleraceus	Asteraceae	Annual	1	1
Tolpis barbata	Asteraceae	Annual	17	1
Trifolium angustifolium	Fabaceae	Annual	10	1
Trifolium arvense	Fabaceae	Annual	31	2
Trifolium campestre	Fabaceae	Annual	1	1
Trifolium glomeratum	Fabaceae	Annual	13	1
Trifolium subterranean	Fabaceae	Annual	29	5
Rushes and Sedges				
Lomandra filiformis	Lomandraceae	Perennial	26	3
Schoenus apogon	Cyperaceae	Perennial	23	5
Carex appressa	Cyperaceae	Perennial	1	30
Carex inversa	Cyperaceae	Perennial	3	1
Eleocharis acuta	Cyperaceae	Perennial	1	25
Luzula ovata	Juncaceae	Perennial	1	2
Juncus capitatus	Juncaceae	Perennial	1	1

Chapter Three:

Fine-scale variables associated with the presence of native forbs in natural temperate grassland

In this chapter I use a case-control study at individual plant level to compare the influence of fine-scale variables on the relative odds that a native forb will be present vs absent in modified grasslands. I compare data on biomass, identified as highly influential our community level survey (Chapter two), physical vegetation structure and soil-surface condition in the immediate vicinity of a focal native forb plant, with data from random locations within 5m which have no native forbs.

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Abstract

While broad scale threats to floristic diversity in native temperate grasslands are well-documented (e.g., elevated soil nutrients, changes in disturbance regimes and exotic species), fine-scale variables associated with the decline of native forbs have received relatively little attention, even though forb presence at a location depends on the conditions in the immediate vicinity. I conducted a case-control study to determine which fine-scale variables influence the occurrence of native forbs in modified grasslands with reduced physical structure, accumulated litter and established exotic species. I compared vegetation composition and physical structure, living and dead biomass, soil surface condition, and the presence of rocks and logs in small 'case' plots containing a focal native forb, with two proximate 'control' plots not containing any native forbs. The odds of a native forb being present (assuming equivalence in land-use history, soils, landscape position, climate, and propagule presence) was significantly associated with the cover of dead biomass, native and exotic grass, and exotic forbs, in the immediate vicinity. If native forb propagules are present, the occurrence of native forbs in temperate native grassland would benefit from periodic actions that provide open areas between grass and exotic forb cover and remove dead biomass.

Introduction

Biodiversity has declined in native grasslands as a result of agriculture, urbanisation, and altered disturbance regimes (McDougall and Morgan, 2005; McIntyre, 2011; Kiehl *et al.*, 2006; Öster *et al.*, 2009; Howe, 1994). Native forbs are one lifeform that has declined considerably in grassland ecosystems as a result of this disturbance (Tremont and Mcintyre, 1994; Brandt and Seabloom, 2012; Stevens *et al.*, 2010). Forbs are an important component of the floral diversity of native grasslands (Tremont and Mcintyre, 1994; McCain *et al.*, 2010; Pallett *et al.*, 2016); and they contribute to soil protection, water filtration, invasion resistance, ecosystem stability and aesthetic value (Tscharntke *et al.*, 2005; Tilman *et al.*, 2006; Wratten *et al.*, 2012). Native forbs also provide habitat resources for other species, including some which perform ecosystem services such as pollination and biological pest

control (Schmidt-Entling and Döbeli, 2009; Williams *et al.*, 2015). Thus, the conservation and restoration of forb diversity in natural grasslands is a topic that has garnered considerable interest (Hobbs *et al.*, 2013; Foley *et al.*, 2005; Suding, 2011).

While broad scale threats to native forbs are well-documented, the influence of fine-scale environmental variables on the likelihood of forb presence has received relatively little attention, even though individual grassland plants respond primarily to conditions in their immediate vicinity. The loss of native forbs from grassland is often associated with increased soil fertility (Dorrough and Scroggie, 2008; Seabloom *et al.*, 2015; Ceulemans *et al.*, 2013), competition with exotic species (Dawson *et al.*, 2012; Scharfy *et al.*, 2011), and the influence of changed disturbance regimes and grazing on species composition and physical structure (Dorrough and Scroggie, 2008; McIntyre and Tongway, 2005). In turn, physical structure and interstitial gap size affect gap dwelling species, such as forbs and non-dominant grasses, through their influence on resource availability within the gaps (Hellström *et al.*, 2009; Morgan, 1998c; Johnson *et al.*, 2018).

It has long been established that physical structure and gap size influence habitat quality for native forbs in intact natural temperate grasslands, in which grass tussocks create spatially heterogenous interstitial gaps that provide habitat for a high diversity of forb species and smaller grasses (Davies *et al.*, 2005; Morgan, 1998c). Forb habitat quality in gaps is associated with light availability and possibly other resource needs such as temperature and soil moisture variation (Morgan, 2001; Goldberg and Werner, 1983; Morgan, 1997). However, in modified grasslands physical structure and gap size can be reduced, either by moderate intensity agricultural grazing (McIntyre and Tongway, 2005), through litter accumulation in gaps if there has been a change in disturbance regimes (McIntyre and Lavorel, 1994) or through a loss of space to exotic forb species and small grass species (Ulrich *et al.*, 2017; Prober and Thiele, 2005). Moderate intensity agricultural grazing may result in widely-spaced grass tussocks being gradually replaced by closely-spaced small statured species and reduced interstitial gap size (while possibly increasing total gap area)(McIntyre and Tongway, 2005). Such a change in physical structure reduces habitat quality for many forb species because it allows increased light penetration and provides less physical and climatic protection to plants (e.g., forbs) and soil within these

gaps (McIntyre and Tongway, 2005; Diaz *et al.*, 2007; McIntyre, 2005). Morgan (1998c) found that seedling survival of five native forb species declined in gaps with radii less than 18 cm. A layer of dead leaf litter may accumulate in the absence of disturbance to control biomass (e.g., grazing, fire), depending on the rates of litter production and decomposition (Facelli *et al.*, 1991). Litter can restrict forbs through light limitation or physical obstruction (Loydi *et al.*, 2013), affecting native forbs more severely than exotic forb species (Johnson *et al.*, 2018), and exotic forbs and/or grasses compete with native forbs for within-gap resources (Lenz *et al.*, 2003; Morgan, 1998c). I need consider scale when determining the impact of exotic species, as exotic and native diversity are often negatively correlated at fine-scale and positively at larger scales (Fridley *et al.*, 2007), depending on the spatial heterogeneity in environmental conditions (Davies *et al.*, 2005; Price and Morgan, 2010).

I recognize the impacts of land-use history, changed disturbance regimes and exotic species on the heterogeneity of grassland physical structure and community composition, and were interested in the implications of these changes on the variables associated with recruitment niche and fine-scale habitat quality for native forb species. I sought to identify which fine scale variables are associated with the occurrence of native forbs in modified grasslands with a less homogenous physical structure (possibly due to a history of agricultural grazing) accumulated litter, and a widespread presence of exotic forb and grass species. Fine-scale variables directly influence environmental conditions within interstitial gaps, and therefore the plants growing within them – they are also the variables most readily managed. This information will help managers intending to restore forb diversity in modified grasslands where forbs have been lost due to a history of agricultural use or lack of appropriate management.

I also investigated whether these variables affected the occurrence of native forbs primarily through their influence on conditions affecting reproduction (the regeneration niche) (Grime, 1977), or reproduction as well as competition for resources after establishment (Silvertown, 2004; Lourens Poorter, 2007). The regeneration niche may be affected by physical obstruction of seed arrival or seedling emergence (e.g. litter), physical structure providing shelter for germination and seedling survival (e.g. tussock size, gap

size) and/or by plants competing with seedlings for resources (e.g. light, moisture and nutrients). Variables associated with competition for resources (e.g. light, moisture and nutrients) also influence the survival of adult plants. Observational studies of native grasslands usually focus at a plot or broader scale to investigate these issues (Seabloom *et al.*, 2015; Sebastiá, 2004), but I decided that a field-based case-control study at the scale of individual plants would be more informative for native forbs which may be widely scattered in modified grasslands.

Methods

Study area

Our study was undertaken in an area of modified temperate grassland within approximately 70 hectares of grassy woodland within a recently declared nature reserve in the Australian Capital Territory (ACT) in south-eastern Australia (35.270562^{o} S, 149.026425^{o} E). Prior to its declaration as a nature reserve in 2010, the site had a history of grazing by sheep and cattle. The study area is approximately 580 m above sea level, has a median annual rainfall of 650 mm and a mean minimum and maximum daily temperature of 7.0^{o} and 20.8^{o} Celsius (http://www.bom.gov.au/climate/data/index.shtml). Soils in the study area have low to moderate fertility (King, 1996), except where super-phosphate was applied with *Avena sativa* and *Trifolium subterraneum* seed sown from as early as 1947 (ACT Govt. Environment and Planning Directorate, TL2432#01 - Department of the Interior - Property and Survey Branch. Block 21 Belconnen - O.H. Dixon). Vegetation in the ground-layer ranges from areas dominated by exotic grasses and forbs to areas dominated by native species. There is on-going low intensity kangaroo and rabbit grazing, managed by culling. A portion of the site was affected by fire in 2003, and areas of St John's Wort (*Hypericum perforatum*) infestation were boom-sprayed with a non-residual selective broadleaf herbicide (fluroxypyr) in 2011 and 2012.

Data collection

I employed a case-control design—a method originally developed to identify causes of rare diseases (Mann, 2003)—to examine fine-scale variables associated with the occurrence of individual forbs. This approach was preferred to random sampling due to the tendency of

native forbs to be sparsely distributed in modified grasslands (Keating *et al.*, 2004). To our knowledge this method has not been previously used in studies of grassland forbs.

I identified 145 case plots, each with two associated control plots (Fig. 3.1). The key difference between case and control plots being the presence of a native forb at the centre of case plots (control plots contained no native forbs). To identify case plots, I searched for native forbs of any species within a matrix of circular search zones with radii of 20 m at 60 m intervals along parallel transects 60 m apart. I commenced zone searches in the centre and spiraled outwards until a native forb was encountered or the radius distance of 20 m from the centre was reached. If a native forb was located, I marked the centre of a circular case plot. For each case, I located potential control plot centres at random distances (within 1-5 m) and compass bearings from the case plot centre until I found two points with no native forb species within a radius of 30 cm. These points marked the centres of the control plots. Proximity of case and control plots minimises between-plot variation in land-use history, soil properties (texture and fertility), landscape position, distance to (and population size of) forb propagule sources (native and exotic), and climatic variables, such that differences in forb presence/absence are likely to be associated with variables that may vary over short distances.

All fieldwork was undertaken during austral late spring/early summer of 2013. I acknowledge that differences in phenology (growing season) may influence the competitive pressure influenced individual grass species, depending on the time of year, but we conducted the survey during spring, when the majority of grassland species are actively growing and total competitive pressure is high. Therefore, even though I acknowledge there are limitations in what a single survey can reveal, I believe that the results accurately identify the fine-scale variables with the greatest influence on native forb presence.

I measured variables that: (i) directly influence the regeneration niche for native forbs (soil moisture, temperature and hardness, light penetration, %bare ground, %cover of rocks, logs, ground litter and standing dead biomass, litter depth and standing dead biomass height); and (ii) influence both the regeneration niche and the level of competition for resources, affecting the survival of established forbs (grass and exotic forb cover; distance

to, canopy width, and height of tussocks; cryptogam cover, including lichens, mosses, liverworts, fungi and algae)(Fig. 3.1, Table 3.1).

Soil surface conditions (soil moisture, temperature and hardness), which influence forb germination and seedling survival (Morgan, 1998a; Prober *et al.*, 2014; Pennington *et al.*, 2017; Fay and Schultz, 2009) as well as resource availability for other gap dwelling species, may be affected by physical tussock structure and the presence of rocks, logs (Goldin and Hutchinson, 2015) and cryptogams (Eldridge, 1993). However, landscape position (e.g. high or low, drainage or ridge) may also influence soil moisture and temperature and may have greater influence on the spatial distribution of plant species composition and abundance. Light penetration and bare ground also influence microsite suitability for germination and young seedlings, and provide alternate metrics associated with gap size and grass cover (Morgan, 1998c). Ground litter also influences the regeneration niche, through interference during seed dispersal (Ruprecht and Szabó, 2012), and seedling emergence (Loydi *et al.*, 2013), and standing dead biomass affects seedling survival primarily through light restriction (Carson and Peterson, 1990). Grass and exotic forb cover influence the regeneration niche through light restriction and the level of competition affects forbs throughout their life (Adler and HilleRisLambers, 2008; Staples *et al.*, 2016; Gunton and Kunin, 2007). The average distance to and size of grass tussocks influence the regeneration niche through their influence on shelter they provide to forb seedlings, and competition they exert on forbs of any age (Goldberg and Werner, 1983; Morgan, 1998c). Cryptogam cover can also influence seedling recruitment by altering soil surface conditions, and the on-going growth and survival of seedlings by competing for resources (Chamizo *et al.*, 2012).

Most variables (except distance to and size of tussocks) represent observations within a circular quadrat of radius 30 cm (area 0.28 m^2) from the identified plot centres (Fig. 3.1). I used a modified point-centred quadrant technique (Dix, 1961) to derive metrics representing the average distance to, size of, and gap between grass tussocks within 50 cm from the plot centre (Fig. 3.1). A tussock was any grass with a height and/or canopy width ≥10 cm. If no tussocks existed within 50 cm in a quadrant I assumed a default tussock distance of 60 cm; the use of a larger default distance was preferred instead of possibly

calculating the average over less than four quadrants (which would disproportionately weight the importance of tussock distance in the quadrants containing tussocks). Sensitivity testing of alternate default tussock distances of 80 and 100 cm had negligible effect on the results. All surveying (including physical measurements and cover estimations) were carried out by one person to minimize the variation in data that may occur if the surveying was done by multiple field assistants.

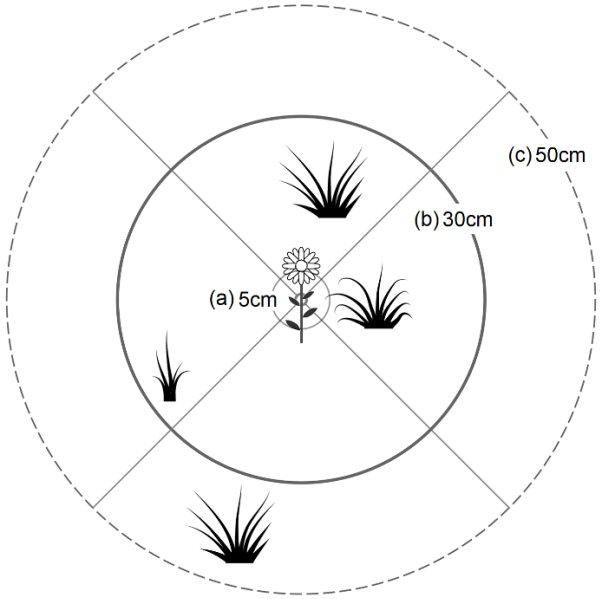

Fig. 3.1. The dimensions of case and control plots used in this study. Case plots were centred on a native forb. Control plots were centred on a point located randomly within 1-5 m of each case provided they did not contain a native forb. For each case and control plot I measured: (a) soil surface condition (moisture, temperature, hardness) within 5 cm of the centre; (b) grass and exotic forb cover, litter depth and cover, standing dead biomass height and cover, and cover of rocks, coarse woody debris and cryptogams within the 30 cm of the centre; and (c) the distance to, height and canopy width of the nearest grass tussock of minimum size (height and/or canopy width ≥10 cm) within 50 cm of the centre (in each of four quadrants).

Table 3.1. Definition and collection method of potential explanatory variables measured in this study.

Explanatory variable	Definition	Collection method
Variables influencing the regeneration niche		
%Soil Moisture	Percentage soil moisture by volume to a depth of 6 cm	Measured with Delta-T Theta Probe ML2X in a gap between plants within 5 cm of the plot centre
Soil temperature	Soil temperature in centigrade at 6cm deep	Measured with Milwaukee TH310 temperature probe in a gap within 5 cm of the plot centre
Soil hardness	Soil surface hardness (0-5 $kgf.cm^2$)	Measured with Controls brand 16-T0171 hand-held soil penetrometer in an undisturbed gap within 5 cm of the plot centre
%Light to ground	Percentage of the above-canopy photosynthetically active radiation (PAR) reaching the ground	Calculated from PAR above the canopy, using a LI-COR LI-191 line quantum sensor, divided by PAR at ground level
%Bare ground	Percentage area of bare ground	Visual estimation of the percentage area of bare ground within a circular plot with 30 cm radius
%Rocks	Percentage of area covered by rocks	Visual estimation of the percentage area covered by rocks within a circular plot with 30 cm radius
%Logs	Percentage of area covered by course woody debris	Visual estimation of the percentage area covered by course woody debris within a circular plot with 30 cm radius
%Litter cover	Percentage of area covered by dead plant material lying on the ground	Visual estimation of the percentage area covered by ground litter within a circular plot with 30 cm radius
Litter depth	Litter depth in cm	Average of 3 random measurements within a circular plot with 30 cm radius
%Dead biomass cover	Percentage of area covered by dead plant material still standing	Visual estimation of the percentage of standing dead biomass within a circular plot with 30 cm radius
Dead biomass height	Height of standing dead biomass in cm	Average of 3 random measurements within a circular plot with 30 cm radius
Variables influencing the regeneration niche and competition for resources		
%Grass cover	Percentage of area covered by living grasses	Calculated based on visual cover estimations of each species in a circular plot with 30 cm radius
%Exotic forb cover	Percentage of area covered by exotic forb species	Calculated based on visual cover estimations of each species in the plot
Tussock distance	Average distance to nearest tussocks in 4 quadrants	Average distance from the focus forb (or plot centre) to the nearest tussock greater than 10 cm in canopy diameter and/or height in 4 point-centred quadrants (PCQs) within 50 cm of the plot centre
Tussock height	Average height of nearest tussocks in 4 quadrants	Average leaf height of the nearest tussock in 4 quadrants within 50 cm of the plot centre
Tussock diameter	Average canopy diameter of nearest tussocks in 4 quadrants	Average canopy diameter of the nearest tussock in 4 quadrants within 50 cm of the plot centre
%Cryptogams	Percentage of area covered by cryptogams	Calculated based on visual cover estimations within a circular plot with 30 cm radius

Data analysis

I examined associations between the occurrence of individual native forbs and the potential explanatory variables (Table 3.1) using conditional logistic regression (Keating *et al.*, 2004) implemented by the "clogit" function within the "survival" package (Therneau 2015) in R (R Core Team, 2016). I included the 15 non-correlated variables ($r < 0.6$) (all variables in Table 3.1 except %Light to ground and Dead biomass height) to identify significant terms ($p < 0.05$) and their odds ratios. The parameter coefficients for the explanatory variables are logarithms of odds ratios, which when exponentiated, represent the change in the relative odds of a native forb being present (i.e. the probability of native forb presence divided by the probability of native forb absence) if an explanatory variable increases by one unit (Monahan *et al.*, 2007). For example, an odds ratio for the variable Litter depth of 0.88 means that the odds of a native forb being present is expected to fall, on average, by 12% with every cm increase in litter depth while an odds ratio for Litter depth of 1.12 means that the odds of a native forb being present is expected to increase by 12% with every cm increase in litter depth. Potential models were ranked using the "dredge" function from the "MuMIn" package (Barton 2017) and selected according to the Bayesian Information Criterion (BIC).

Results

I surveyed 145 case plots, centred on 27 different species of native forbs (Table S3.1 in Appendix S3.1) and 290 associated control plots. The mean distance between the centres of case plots and their matched pair of control plots was 2.89 m (± SD 1.05 m). The species list for case and control plots included eight exotic grass species, 10 native grass species, 22 exotic forb species, 31 native forb species, and more than five other species, mainly sedges and rushes (Table S3.1 in Appendix S3.1). In addition to these, two exotic grasses, two native grasses, and two native forbs were identified at genus level. The case and control plots contained an average of 31% native grass cover, 6% exotic grass cover and 5% exotic forb cover. Summary statistics for all potential explanatory variables and significant correlations are provided in Table 3.2.

Table 3.2. Summary statistics of all data variables collected. Variables with the same superscript letter are significantly correlated ($|r| > 0.6$).

	Variable	Mean	Std Dev	Min	Max
	%Soil Moisture	7.16	4.10	0.2	24
	Soil temperature	24.69	4.10	4.80	38.6
	Soil hardness	3.03	1.36	0.25	5.1
	%Light to ground [a,b]	0.40	0.30	0	1
	%Bare ground	1.54	6.36	0	75
	%Rock cover	0.17	1.65	0	25
	%Logs cover	0.17	1.43	0	20
	%Litter cover	33.50	19.83	0	90
	Litter depth [a]	2.05	1.32	0	6
	%Dead Biomass cover [b,c]	10.30	16.46	0	85
	Dead Biomass height [c]	5.52	5.59	0	38
	%Grass cover	36.84	20.93	0	100
	%Exotic Forb cover	5.19	9.55	0	60
	Tussock distance	28.72	13.64	4	60
	Tussock height	11.82	5.83	0	55
	Tussock diameter	13.78	5.22	0	49
	%Cryptogam cover	0.15	1.00	0	12
#	*%Native Forb cover*	18.42	18.00	1	80

Case plots only

The best conditional logistic model (lowest BIC) selected to predict the odds of a native forb being present contained the following explanatory variables: %Bare ground cover, %Ground litter cover, %Dead biomass cover, %Grass cover and %Exotic forb cover

(Table 3.3). Three of these variables (%Bare ground cover, %Ground litter cover and %Dead biomass cover) are associated with the regeneration niche for forbs and two (%Grass cover and %Exotic forb cover) are associated with the regeneration niche for forbs and competition for resources. The selected model indicated that, for each percentage increase in bare ground cover, ground litter cover, dead biomass cover, grass cover or exotic forb cover, the odds that a native forb will be present declined by a mean of 10-13% (Table 3.3). Delta-BIC values calculated for models in which individual terms were dropped suggested that %Litter cover, %Grass cover and %Dead biomass cover had the greatest influence on the odds that a native forb was present, with %Bare ground cover the least influence (Table 3.3).

Table 3.3. Terms in the best conditional logistic regression model used to predict the odds that a native forb is present. Estimates for each variable, standard errors, statistical significance, and model BIC. Delta-BICs (relative to best-model BIC = 217.7) indicates the change in BIC that would occur if a single variable is omitted from the model.

Variable	Estimate	Std Error	P-value	Odds ratio	95% confidence interval for odds ratio	Delta-BIC
%Bare ground	-0.104	0.026	< 0.001	0.901	0.878 - 0.925	+ 7
%Litter cover	-0.122	0.018	< 0.001	0.885	0.869 - 0.901	+ 92
%Dead biomass cover	-0.125	0.020	< 0.001	0.883	0.865 - 0.901	+ 70
%Grass cover	-0.101	0.016	< 0.001	0.904	0.890 - 0.918	+ 68
%Exotic Forb cover	-0.135	0.028	< 0.001	0.874	0.850 - 0.898	+ 36

Discussion

I sought to identify which fine scale variables are associated with the occurrence of native forbs in grasslands providing a range of environmental conditions and whether these variables affect the occurrence of native forbs through their influence on reproduction (the regeneration niche) (Grime, 1977), or both reproduction and competition for resources after establishment. The odds of native forb being present was associated with variables influencing the regeneration niche (litter cover, standing dead biomass cover, bare ground, grass cover, exotic forb cover) and variables influencing the regeneration niche as well as competition to individual native forbs of all ages (grass cover, exotic forb cover). Distance to, height and canopy width of grass tussocks with height/canopy greater than 10 cm had little influence on the occurrence of native forbs in our study, suggesting that physical structure and gap size between tussocks are less influential than biomass cover on native forb occurrence in modified grasslands.

Ground litter and standing dead biomass were strongly negatively associated with the presence of native forbs through their effect on the regeneration niche (Kelemen *et al.*, 2013; O'Halloran *et al.*, 2013). Ground litter affects the regeneration niche by obstructing the arrival of seed (Ruprecht and Szabó, 2012) and seedling emergence (Letts *et al.*, 2015; Johnson *et al.*, 2018). Negative correlations between light reaching the ground, and litter depth and percentage dead biomass cover (r = -0.68 and -0.61 respectively), suggests that the reduction of light may be one mechanism for the negative effects of litter and dead biomass on native forbs. Light affects germination of some native forb species (Morgan, 1998a), and early survival (Borer *et al.*, 2014; Hautier *et al.*, 2009) of forbs. Although litter can have a positive effect on seedling survival through the retention of soil moisture (Loydi *et al.*, 2013), available soil moisture can also increase the likelihood that native forbs are replaced by species in other functional groups (Tzialla *et al.*, 2006), such as exotic plant species. The amount of litter and dead biomass depends on the dominant grass species and how productive they are, the history of biomass-reducing disturbance, and the rate of decomposition (O'Halloran *et al.*, 2013; Facelli *et al.*, 1991).

The cover of living grass and exotic forbs were also negatively associated with the presence of native forbs. Living grasses and exotic forbs can affect the regeneration niche

of native forbs and their persistence through competition for space and resources (light, moisture, nutrients) (Goldberg and Barton, 1992; Staples *et al.*, 2016; Johnson *et al.*, 2018). The occurrence of gap-dwelling forbs is likely to be limited by grasses competing for available soil nutrients and/or moisture, as light will be relatively abundant and soil moisture less protected from evaporation, depending on the height and openness of the canopy (Borer *et al.*, 2014). Native forbs also compete with gap-dwelling exotic forbs (Suding and Goldberg, 2001; Loydi *et al.*, 2015), and the arrival of exotic species is often associated with a decline in native species richness (Brandt and Seabloom, 2012).

The cover of bare ground had a minor influence on the occurrence of native forbs. Interstitial gaps (between grass tussocks) are usually considered essential fine scale habitat for subordinate species such as forbs (Hellström *et al.*, 2009; Morgan, 1998c). However, in our study the cover of bare ground (representing the total area of interstitial gaps minus the area occupied by litter and gap-dwelling plants) was negatively associated with the occurrence of native forbs. This result possibly reflects a change in the size-class distribution of interstitial gaps in our study area (i.e. a large number of small gaps), possibly due to previous livestock grazing (McIntyre and Tongway, 2005). Small gaps may have a negative effect on survival if they are too small to isolate forb seedlings from competition (Morgan, 1998c). Tussocks of minimum size were uncommon in our study area, possibly due to a history of livestock grazing (McIntyre and Tongway, 2005) and areas with low productivity.

While a diversity of responses to environmental conditions is likely among native forb species (Clarke and Davison, 2004; Silvertown, 2004), our results support other research (Letts *et al.*, 2015; Loydi *et al.*, 2013; Johnson *et al.*, 2018) finding that the occurrence of native forbs is less likely in the presence of a high cover of dead biomass, grass and exotic forbs. The rate of dead biomass accumulation is usually higher in productive areas but can be difficult to predict because decomposition rates may vary for reasons not fully understood (O'Halloran *et al.*, 2013; Facelli *et al.*, 1991). Where litter accumulates (i.e., productivity exceeds decomposition rates), reductions in dead (and living) biomass may be possible with appropriate use of fire, but burning may not be possible near built-up areas, and may not be beneficial under adverse seasonal conditions

(affecting the ability to burn, or fire intensity) (Prober *et al.*, 2007; Morgan, 2015). Similar reductions in grass and forb cover may be achieved with mowing or light grazing. However, mowing should be followed by the removal of cut material (e.g. by raking) or it will add to dead biomass cover; and medium-high intensity grazing should be avoided as it may risk further decline of native forbs due to impacts on physical structure, trampling or soil compaction (Verrier and Kirkpatrick, 2005; Dorrough and Scroggie, 2008; McIntyre and Tongway, 2005). Periodic management of living and dead biomass should increase the likelihood that native forb populations will recover or expand, but the success of these actions depends on an adequate supply of viable propagules. Native Australian forbs do not form a long-lived seed bank (Morgan, 1998b) so, unless there is a population of native forbs present on or very near the site, seed or seedlings of species suited to the location will need to be introduced.

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Appendix S3.1

Table S3.1. Species list of recorded plants, including longevity, the number of plots and average cover.

Species	Longevity	Plots	Avg Cover
Grasses - Exotic			
Aira spp	Annual	31	7
Avena sativa	Annual	46	17
Briza minor	Annual	13	5
Bromus diandrus	Annual	30	14
Bromus hordaceous	Annual	108	4
Cynosurus echinatus	Annual	2	1
Holcus lanatus	Annual	5	3
Vulpia spp	Annual	59	7
Lolium perenne	Perennial	8	2
Phalaris aquatica	Perennial	6	21
Grasses - Native			
Austrostipa bigeniculata	Perennial	44	15
Austrostipa scabra	Perennial	44	12
Bothriochloa macra	Perennial	18	14
Dichelachne spp	Perennial	1	25
Elymus scaber	Perennial	25	3
Eragrostis brownii	Perennial	4	5
Microleana stipoides	Perennial	107	18
Panicum effusum	Perennial	4	4
Poa labilladieri	Perennial	1	5
Poa sieberiana	Perennial	22	13
Rytidosperma spp	Perennial	59	8
Themeda australis	Perennial	311	30
Forbs - Exotic			
Centaurium erythraea	Annual	1	1
Conyza bonariensis	Annual	18	3
Echium plantagineum	Annual	2	3
Hypochaeris glabra	Annual	38	4
Lactuca serriola	Annual	14	5
Petrorhagia nanteuilii	Annual	28	5
Plantago lanceolata	Annual	30	6
Sonchus oleraceus	Annual	2	1
Tolpis barbata	Annual	8	2
Tragopogon dubias	Annual	6	3
Trifolium angustifolium	Annual	1	1
Trifolium arvense	Annual	9	7
Trifolium glomeratum	Annual	2	1
Trifolium subterranean	Annual	10	11
Carthamus lanatus	Annual	4	3
Cirsium vulgare	Annual	24	8
Acetosella vulgaris	Perennial	33	11
Chondrilla juncea	Perennial	5	6
Hypericum perforatum	Perennial	75	10
Hypochaeris radicata	Perennial	28	9
Forbs - Native (Case plots)			
Euchiton sphaericus	Annual	1	40
Triptilodiscus pygmaeus	Annual	5	3
Hypericum gramineum	Either	5	4
Vittadinia cuneata	Either	6	29
Acaena ovina	Perennial	7	20
Asperula conferta	Perennial	3	8
Bulbine bulbosa	Perennial	11	8
Cheilanthes spp	Perennial	6	15
Chrysocephalum apiculatum	Perennial	14	29
Convolvulus erubescens	Perennial	3	5
Cymbonotus preissianus	Perennial	9	9
Desmodium varians	Perennial	1	3
Dichondra repens	Perennial	4	25
Dichopogon fimbriatus	Perennial	3	10
Eryngium ovinum	Perennial	6	12
Euchiton gymnocephalus	Perennial	1	20
Gonocarpus tetragynus	Perennial	1	4
Haloragis heterophylla	Perennial	5	44
Hydrocotyle laxiflora	Perennial	6	23
Leptorhynchos squamatus	Perennial	11	7

Paronychia brasiliana	Perennial	7	9
Sanguisorba minor	Perennial	1	20
Rushes & Sedges			
Lomandra filiformis	Perennial	24	8
Lomandra longifolia	Perennial	1	15
Carex inversa	Perennial	11	8
Juncus filicaulis	Perennial	2	2
Other sedge		14	4
Other			
Rubus spp	Perennial	1	8
Oxalis perennans	Perennial	2	23
Plantago varia	Perennial	11	20
Senecio quadridentatus	Perennial	2	9
Solenogyne dominii	Perennial	2	20
Tricoryne elatior	Perennial	6	6
Vittadinia meulleri	Perennial	2	6
Wahlenbergia stricta	Perennial	12	6
Forbs - Native (not found in Case plots)			
Erodium crinitum	Annual	1	5
Euchiton involucratis	Perennial	1	5
Geranium solanderi	Perennial	2	3
Rumex brownii	Perennial	2	16

Chapter Four:

Seed addition and biomass removal key to restoring native forbs in degraded temperate grassland

This chapter explains a controlled field experiment designed to test the results of the studies explained in Chapters two and three, which suggest that biomass is a key factor restricting native forbs. Research suggests that seedling emergence is a highly vulnerable phase of plant recruitment, so I measured seedling emergence responses of native seedlings (from sown seed) and exotic seedlings to treatments manipulating the amount of living and dead biomass. I also measured the resulting changes in vegetation structure and resource variables to identify the continuous variables influencing seedling emergence.

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Johnson, D. P., Catford, J. A., Driscoll, D. A. and Gibbons, P., 2018. Seed addition and biomass removal key to restoring native forbs in degraded temperate grassland, Applied Vegetation Science, 21(2): 219-228. Available at: doi:10.1111/avsc.12352

Abstract

Questions

Long-term restoration of native forb diversity can only be achieved if native forb species can recruit (colonise and establish) and reproduce. I asked whether native forbs in a temperate grassland were seed limited, and how the recruitment of native and exotic forbs is affected by grassland structure and resource availability.

Location

Australian Capital Territory, south-eastern Australia.

Methods

I conducted a field experiment in a temperate grassland dominated by a native tussock grass to assess effects of: 1) addition of native forb seed, 2) thinning of native grass tussocks, 3) leaf litter removal, and 4) exotic plant removal on the recruitment of native and exotic forbs. These four actions can alter grassland physical structure and the availability of soil nutrients, soil moisture, and light. I used generalised linear mixed models to determine the importance of seed addition, grassland structure and resource availability on the richness and abundance of sown native forbs, and the abundance of exotic forb seedlings and unsown native forbs.

Results

Adding seed increased the species richness and abundance of native forbs. Tussock thinning and litter removal increased species richness and abundance of sown native forbs, and the abundance of exotic forb seedlings. Exotic plant removal also increased the abundance of sown native forbs. Abundance of unsown native forb species was unaffected by the experimental treatments. Species richness and abundance of native forbs and abundance of exotic forbs declined with increasing tussock grass cover. Leaf litter restricted the abundance of native forb species more than exotic forb species.

Conclusion

Native forb recruitment predominantly relied upon seed addition, suggesting that seed limitation is a major barrier to the recovery of degraded grasslands. Reducing the cover of living grass tussocks facilitated recruitment of native and exotic forbs, and removing litter disproportionally increased recruitment of native forbs compared with exotics. Combining seed addition with the reduction of both living and dead grass biomass should help restore native grassland forbs.

Introduction

Forb diversity has declined in many parts of the world following the conversion of grasslands for agriculture, and often remains low even when agriculture is subsequently abandoned (Fensham *et al.*, 2016; Wheeler *et al.*, 2015). Forbs represent a large proportion of plant species richness in natural grasslands (Jacquemyn *et al.*, 2011; Klimek *et al.*, 2007; Mitchell and Bakker, 2016; Tremont and Mcintyre, 1994) and their decline reduces the functional diversity of grassland ecosystems (Hooper *et al.*, 2005). The decline of grassland diversity has flow-on impacts on pollinator diversity (Wilkerson *et al.*, 2014), resistance to invasion (Hulvey and Zavaleta, 2012), and the availability of habitat resources for other grassland dependent taxa (Barrett *et al.*, 2008). Restoring and maintaining forb diversity is thus a key conservation goal for grasslands around the world (Hobbs *et al.*, 2013). The Australian Government lists the ecosystem under consideration in this study as Critically Endangered, largely on the basis of lost forb diversity (https://www.environment.gov.au/system/files/resources/be2ff840-7e59-48b0-9eb5-4ad003d01481/files/box-gum.pdf; accessed 12-10-2017).

Low rates of seedling emergence and survival may explain why few forb species recover once agriculture has ceased (Fayolle *et al.*, 2009; Donath and Eckstein, 2010). Poor seedling emergence and survival can result from limited availability of both seeds and other resources that are needed for early survival and growth (Zobel *et al.*, 2000; Brandt and Seabloom, 2012; Dybzinski and Tilman, 2012). Seed supply may be limited because of an absence of source populations, poor dispersal from source populations, and the lack of a persistent soil seedbank (Brandt and Seabloom, 2012). If forb species are seed-limited, increasing seed availability (e.g. through direct seeding) will be required (Jacquemyn *et al.*, 2011; Morgan and Williams, 2015). Even where adequate seed is present, recruitment requires resources essential for plant growth (light, nutrients and moisture), which may be influenced by grassland structure and competition (Morgan, 1998c; Hellström *et al.*, 2009; Frances *et al.*, 2010).

Understanding the ways in which different management actions affect native and exotic forb recruitment will help optimise restoration (Fig. 4.1). For example, the actions needed to restore native forb diversity will differ if they are physically restricted by

accumulated leaf litter, or if they are resource-limited due to competition from established vegetation (Moles and Westoby, 2004). If restricted by litter or competition from native grasses, litter removal and control burns would be effective management actions (Fynn *et al.*, 2005), whereas weed control would be more effective in the case of restriction by exotic species (Prober and Thiele, 2005). Understanding how exotic forbs respond to management is also important because management actions designed to increase native forb recruitment may also inadvertently facilitate exotic species recruitment (Faithfull *et al.*, 2012; Driscoll and Scheiner, 2017). I compare native and exotic forb seedling emergence under treatments designed to manipulate physical structure and resource availability. In addition, I measure physical structure and resource availability to investigate the degree to which microsite suitability for forb recruitment is associated with a species' evolutionary adaptations to resource availability in its region of origin.

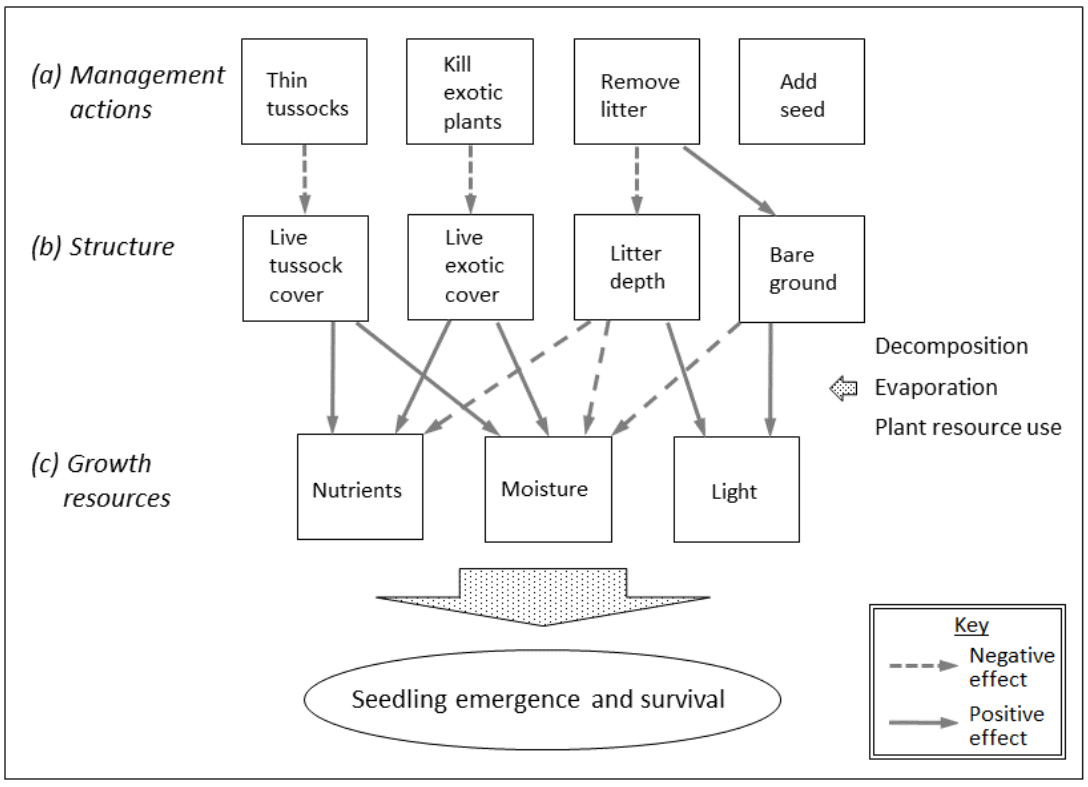

Fig. 4.1. Hypothesised impacts of management actions on grassland structure, resource availability, and native forb recruitment. Management actions (a) change grassland structure (b), and structural changes influence resource availability (c) by initiating changes

in the rates of decomposition, evaporation and plant resource use. The changes flowing from each of these management actions are hypothesised to have a positive effect on seedlings.

Temperate grasslands in south-eastern Australia exemplify the challenges faced when attempting to restore forb diversity in grasslands. Agriculture practices over the last 200 years have drastically simplified Australian grasslands, with fertiliser application, herbicide use, sowing of exotic pasture grasses, livestock grazing, and suppression of fires all being common practice. As a result, less than 0.5% of the original extent of diverse grassland ecosystems remains (Prober *et al.*, 2013), and remnant grassland is now isolated in small fragments (Tremont and Mcintyre, 1994), limiting opportunities for seed dispersal among patches.

In this experimental study, I implemented four treatments (thin tussocks, kill exotic species, remove litter, and add seed) that represent key management actions for restoring grassland forbs. Our aims were to: i) assess the necessity of seed addition for the re-establishment of native forbs, ii) determine which treatments, structural components and resources are associated with native forb recruitment (Fig. 4.1), and iii) compare how the abundance of native forbs and exotic forbs respond to these treatments.

Methods

Study site

I conducted the experiment in a small area of temperate grassland in the Australian Capital Territory (ACT) in south-eastern Australia (35.270562^{o} S, 149.026425^{o} E, 574 m above sea level). The site was located in a nature reserve, and has a median annual rainfall of 650 mm. The 1.5 km^{2} reserve was previously a pastoral lease starting with sheep grazing from the 1920s and more recently (1985-2005) low intensity cattle grazing. It was declared a nature reserve in 2010. In 2011 and 2012, a non-residual selective broadleaf herbicide (fluroxypyr) was applied (via boom-spraying) in parts of the reserve to control (successfully in the short-term) an invasive exotic forb, *Hypericum perforatum*.

I established the experiment on a gentle (5 degrees), south-facing slope that was dominated by *Themeda triandra* (Kangaroo grass) before the experiment began. *Themeda*

triandra is a summer growing native perennial tussock grass, which can become dominant when present. In productive conditions, *T. triandra* accumulates a thick mat of dead leaf litter—from leaves that die over winter—if not removed (e.g. by fire). The litter was up to 3 cm deep in parts of the study site, with an average dry mass of 310 $g.m^{-2}$. Before the start of the experiment, 43 native forb species (up to 10 species per 0.5 m^2) were observed across the whole reserve (Johnson 2013, Unpubl.), but only 10 forb species (up to 4 species per 0.5 m^2) were observed in the experimental site, probably due to previous herbicide use and lack of disturbance. Exotic grasses and forbs are abundant in the surrounding area (>2 m from experimental site), but made only a minor contribution to vegetation in the experimental site.

Experimental design

The experiment was a fully crossed factorial design with all 16 (2^4) treatment combinations randomly arranged within each of six blocks, making a total of 96 plots (Table S4.2 in Appendix S4.1). The plots, measuring 0.75 m x 0.75 m and separated by at least 75 cm, were located within a single 1000 m^2 area of homogenous grassland to minimise variation due to topography, soils, weather, and vegetation type. The area was fenced to minimise grazing by vertebrate herbivores (kangaroos, rabbits).

The experimental plots within each block were randomly treated with every combination of: (a) *T. triandra* tussocks thinned by ~50% by spraying with glyphosate (7.2 g.L), (b) all leaf litter on the ground removed by hand, (c) all exotic grasses and forbs killed by painting individuals with glyphosate (7.2 g.L), and (d) addition of seed for 14 native forb species that occur naturally in the region (Tables S4.1 and S4.2 in Appendix S4.1). Three of the sown species already occurred in the site. Plants killed with glyphosate were not removed. All treatments were applied in the austral spring (5-6 Oct 2014), and exotic plant removal was done three more times: late-spring (Nov 2014), summer (Jan 2015) and early autumn (Mar 2015). The plots were watered with a known amount applied evenly using a spray nozzle and a timer as required to encourage germination in the first month, and after that at a rate equivalent to the 75^{th} percentile of historic rainfall to simulate a good growing season.

Response variables

The four response variables were: i) the species richness of sown native forbs (age < 8 months); ii) the abundance (count of individuals) of sown native forbs; iii) the abundance (count) of unsown exotic forbs; and iv) the abundance (count) of unsown native forbs (Table S4.3a in Appendix S4.1). Response variables were measured in the central 0.5 m x 0.5 m of each 0.75 m x 0.75 m plot to avoid edge effects. For sown native forbs, I used the maximum counts from two surveys conducted in early summer and early winter, 11 weeks and eight months after the treatments, respectively, as the best indication of total recruitment over that period. Count averages were not possible because of difficulty determining the season in which individuals had emerged. I did not measure the species richness of exotic forbs as individuals were too small to identify to species-level (Table S4.6c in Appendix S4.2).

Explanatory variables

I measured six potential explanatory variables to characterise physical structure and resource availability within each plot.

Physical structure within the central 0.5 m x 0.5 m of each plot was represented by i) the percentage area covered by living grass tussocks, not including attached tussock biomass that had senesced due to the thinning treatment or natural die-back over winter; ii) ground litter depth averaged from three measurements; and iii) the visually estimated percentage area of bare ground (Table S4.3b in Appendix S4.1). These data were recorded separately for each quarter of a 0.5 m x 0.5 m quadrat, and the results subsequently averaged. I did not include the cover of exotic species as a structural explanatory variable because exotic species made up little cover (mean 2.2% ± SD 7.1%) prior to the final months of the experiment.

Resource availability was represented by: i) the percentage of light penetrating the canopy to above the litter layer, measured with a LI-COR LI-191 line quantum sensor positioned along both diagonals of the 0.75 m x 0.75 m square plots; ii) available soil phosphorus measured within a $NaHCO_3$ extract of the soil using a Lachat QuikChem 8500 flow injection analyser; and iii) the percentage of soil moisture, by volume, measured in the outer 12 cm on opposing sides of each plot with a Delta-T Theta Probe ML3 moisture

probe four days after rain (Table S4.3b in Appendix S4.1). Soil sampling and moisture measurement was done in the outer 12 cm to avoid disturbance to the central 0.25 m^{-2} where the plant responses and structural variables were measured.

I also measured the oven-dry weight (80^{o} C) of litter lying on the ground—consisting almost entirely of dead tussock leaves—gathered from twelve untreated 0.25 m^{-2} patches, two beside each block, to estimate the overall litter biomass.

Statistical analysis

I used generalised linear mixed models (GLMM) within the "glmmTMB" package in R statistical software (R Core Team, 2016) to model relationships between plant responses to: (a) the experimental treatments, and (b) to explanatory covariates representing physical structure and the availability of resources within each plot that are potentially affected by the treatments. Responses to treatments and covariates were modelled separately as there were strong correlations between the tussock thinning treatment and the percent cover of living tussock (r = -0.65), and between the litter removal treatment and litter depth (r = -0.86). All combinations and subsets of the structural variables (percent cover of living tussock, litter depth, and bare ground) and resource variables (light penetrating the canopy, available soil phosphorus, and soil moisture) were evaluated as potential model terms. Total tussock cover was excluded due to correlation with cover of living tussock (r = 0.82). Light at ground level was excluded due to correlation with light penetrating the canopy (r = 0.72) and bare ground (r = 0.71). The remaining potential explanatory variables were not highly correlated (r < 0.7).

I used a Poisson distribution with log-link function to model sown native forb species richness, and negative binomial distributions for plant abundance data because they were more widely dispersed than Poisson distributions due to high numbers of zeros and several high scores. Of the 96 plots in the experiment, 48 were sown with native forbs. Only data from the 48 sown plots were used to model sown native forb responses, because no sown native forbs were found in the unsown plots. I modelled exotic forb responses using data from the 48 plots where the exotic removal treatment was applied to ensure that counts were of seedlings only. Data from all 96 plots were used to model the response of unsown native forb species.

I fitted block number, representing the individual blocks in which treatments were grouped, as a random effect term to account for variation between blocks. I used Bayesian Information Criterion (BIC) information criteria to determine the fixed terms of the 'best fit' models, out of all possible non-correlated combinations. I calculated *Delta BIC*–the difference in BIC between the 'best fit' and the 'best fit minus one term' models–as a basis for comparing the relative influence of individual terms (Burnham and Anderson, 2003).

Results

I recorded a total of 4465 forb individuals: 1264 seedlings for nine of the 14 sown native forb species (Table S4.6a in Appendix S4.2), 2001 individuals of exotic species (including 900 seedlings in plots where exotic species had been removed), and 1200 unsown native forb individuals of any age (Table S4.6b in Appendix S4.2).

Prior to establishing the treatments, native species richness (mean 1.1 ± SD 1.1) and percent cover of native species (6.2% ± 10.7%) across all plots (not including *T. triandra*) was higher than for exotic species (0.3 ± 0.6, and 2.2% ± 7.1%).

Responses to treatments

There were statistically significant associations between three of the four response variables (sown native forb richness, sown native forb abundance and exotic forb abundance) and one or more of the treatments (Table 4.1, Fig. 4.2). Seedlings of the sown native forb species only emerged where seed had been added, and with no other treatments mean total native richness and abundance increased by 122% and 61% respectively. Where seed was added, mean total richness and abundance of native forbs increased with tussock thinning (+214%, +78%), litter removal (+160%, +64%), or both (+575%, +488%) (Table S4.2 in Appendix S4.1).

Sown native species richness and abundance, and exotic abundance were positively associated with tussock thinning and litter removal. Sown native abundance was also positively associated with exotic species removal (Table 4.1a-c, Fig. 4.2a-c). Litter removal had a greater influence than tussock thinning on the richness (*Delta BIC* = 49 vs 8) and abundance (*Delta BIC* = 41 vs 25) of sown native forbs (Table 4.1a-b). However, litter

removal and tussock thinning had similar influence on the abundance of exotic forbs (*Delta BIC* = 9 vs 8) (Table 4.1c). There were no additional effects from interactions between treatments.

Table 4.1. GLMM models fitted to predict the effects of the experimental treatments on: (a) sown native forb species richness (seeded plots only), (b) sown native forb abundance (seeded plots only) and (c) exotic forb seedling abundance (exotic removal plots only). There were no significant models predicting the response of (d) unsown native forb abundance to the treatments (all plots). Block number was included as a random effect in each model.

Plant group response	BIC	df	Treatment	Estimate	Std. Err.	F pr.	Delta BIC
(a) Sown native forb species richness	204.2	43	Thin tussocks	0.498	0.146	<0.001	8
			Remove litter	1.105	0.163	<0.001	49
(b) Sown native forb abundance	353.6	42	Thin tussocks	1.697	0.201	<0.001	25
			Remove litter	2.284	0.240	<0.001	41
			Remove exotics	0.492	0.149	<0.001	6
(c) Exotic forb abundance	367.9	43	Thin tussocks	1.152	0.311	<0.001	8
			Remove litter	1.212	0.317	<0.001	9
(d) Unsown native forb abundance	N/A						

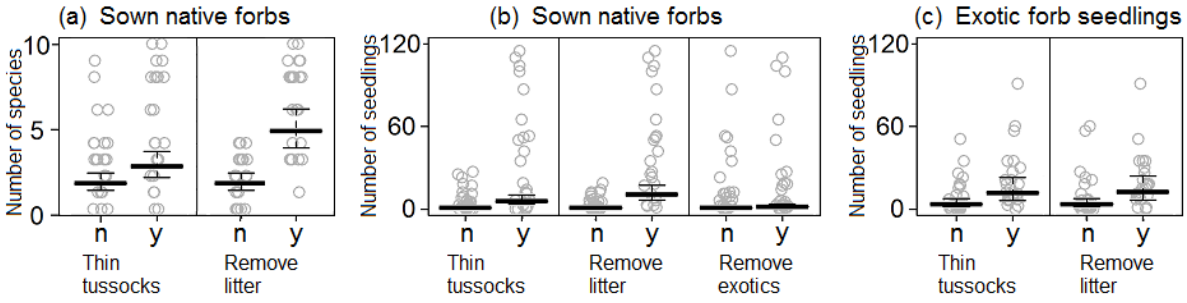

Fig. 4.2. Effects of the three treatments (thin tussocks, remove litter, remove exotics) on (a) sown native forb species richness, (b) sown native forb abundance and (c) exotic forb abundance. Predicted responses (mean ± 95% confidence intervals) to individual treatments (y), assuming all other treatments are controls (n), were obtained from GLMMs in which the block number was fitted as a random effect. Observed values are indicated by open circles [two observed counts not shown – maximum (b)(193) and maximum (c)(188)].

Responses to structure

Sown native species richness and abundance, and unsown native forb abundance were significantly negatively associated with the explanatory variables representing grassland structure (live tussock cover and litter depth). Litter depth had the greatest relative influence on these models (*Delta BIC* = 26, 27 and 5 respectively). In addition, unsown native forb abundance was negatively associated with the percent area of bare ground. Exotic forb abundance was associated with live tussock cover but not litter depth (Table 4.2a-d, Fig. 4.3a-d). Weak correlations between live tussock cover and the availability of the measured resources (light penetrating the canopy, r = -0.38; light at ground level, r = 0.00; phosphorus, r = -0.13; soil moisture, r = -0.24) suggest the effects of live tussock cover cannot be explained by its effect on these resources. A negative correlation between litter depth and light at ground level (r = -0.85) demonstrates a strong shading effect, but weak correlations between litter and the other measured resources (phosphorus, r = 0.00; and soil moisture, r = 0.33) suggested the effect of litter was not because of its influence on these resources.

Table 4.2. GLMM models predicting the responses of: (a) sown native forb species richness (seeded plots only), (b) sown native forb abundance (seeded plots only), (c) exotic forb seedling abundance (exotic removal plots only), and (d) unsown native forb abundance (all plots) to the experimental covariates. Block number was included as a random effect in each model.

Plant group response	Covariate	BIC	df	Estimate	Std. Err.	F Pr.	Delta BIC
(a) Sown native	%Live tussock cover	206.7	42	-0.012	0.005	0.031	1
forb species richness	Litter depth			-0.671	0.134	<0.001	26
	%Light penetrating canopy			0.017	0.007	0.021	1
	Phosphorus			-0.124	0.051	0.014	2
(b) Sown native	%Live tussock cover	371.5	43	-0.044	0.011	<0.001	9
forb abundance	Litter depth			-1.475	0.204	<0.001	27
(c) Exotic forb	%Live tussock cover	352.7	43	-0.022	0.010	0.027	1
abundance	%Light penetrating canopy			0.065	0.012	<0.001	17
(d) Unsown native	%Live tussock cover	686.3	89	-0.016	0.006	0.005	3
forb abundance	Litter depth			-0.530	0.173	0.002	5
	Soil moisture			0.058	0.025	0.019	0

Bare ground	-0.022	0.008	0.007	3

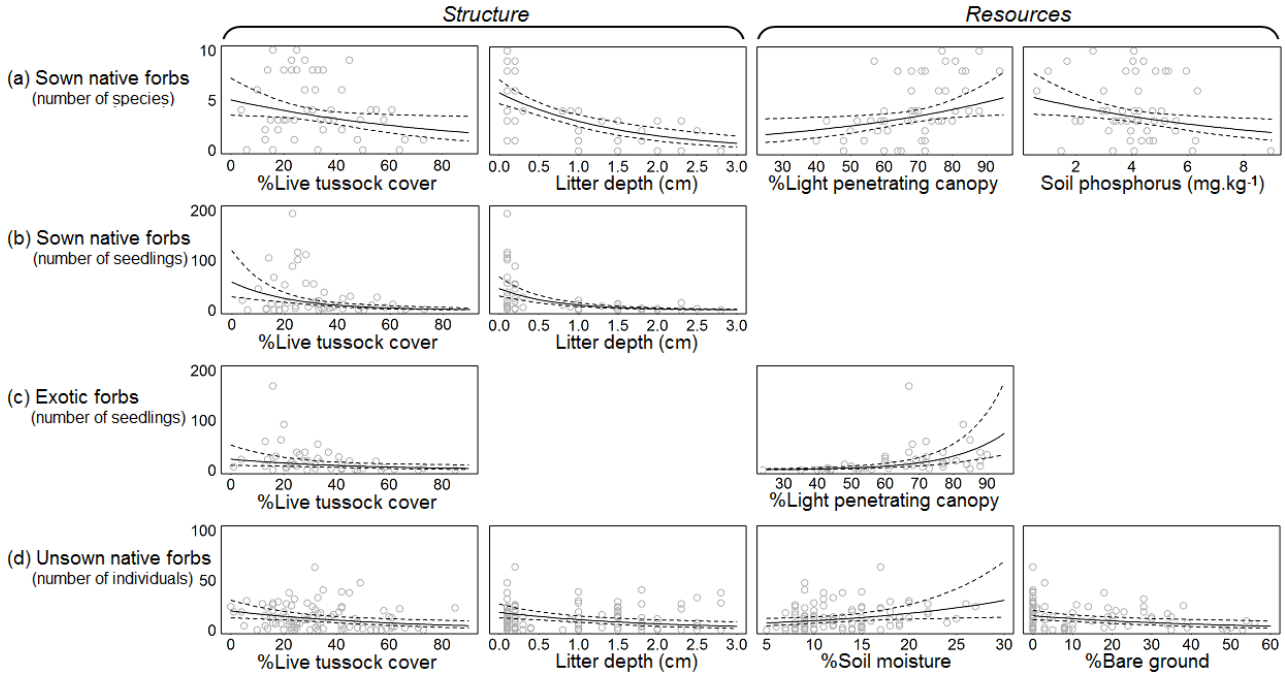

Fig. 4.3. Predicted responses to structural and resource variables (mean ± 95% confidence intervals) from the fitted GLMMs for a) sown native forb species richness, b) sown native forb abundance, c) exotic forb seedling abundance and d) unsown native forb abundance. Predictions were calculated across the range of values observed for each fixed effect in the GLMM with other fixed effects held at their mean. Observed values are indicated by open circles.

Responses to resource availability

Sown native forb species richness was positively associated with the percent light penetrating the canopy and negatively associated with soil phosphorus. Sown native forb abundance was not associated with any of the measured resources. Unsown native forb abundance was positively associated with soil moisture. Exotic forb seedling abundance was positively associated with percent light penetrating the canopy, which had a greater influence on this response than percent live tussock cover (*Delta BIC* = 17 vs 1), the structural variable in that model. In all other models, resource availability had a relatively minor influence compared with structure (Table 4.2a-d, Fig. 4.3a-d).

Discussion

I investigated whether the addition of seed is needed to restore native forbs in a temperate grassland; and compared responses by native and exotic forbs to tussock thinning, litter removal and weed control. I found that the richness and abundance of native forbs were significantly positively associated with the addition of seed, removal of living biomass (native tussock grasses and exotic species) and removal of litter. Our results agreed with studies conducted in other parts of the world that demonstrate the negative influence that competition from established plants and litter can have on forb seedling recruitment (Loydi *et al.*, 2013; Dybzinski and Tilman, 2012; Scott and Morgan, 2012a). I found that native forb seedlings were more restricted by litter than exotic forb seedlings, and thus, controlling litter build-up is essential for maintaining the richness and abundance of native forbs. However, our results indicated that tussock thinning and litter removal will not result in successful establishment of native forb species without an adequate supply of seed.

The need for seed

Seed addition will probably be necessary when restoring forb diversity in degraded temperate grasslands, as there is little potential for restoring lost forb diversity through natural dispersal (Heinken and Weber, 2013; Hobbs and Yates, 2003). While the use of herbicides may have reduced the supply of naturally occurring native forb seed at the experiment site, small and fragmented populations of persisting species are universal symptoms of grassland degradation (Hobbs and Yates, 2003). And lost species above ground are also likely to be lost from the soil seedbank (Morgan, 2001). Clark et al. (2007) argue that the availability of suitable sites is more limiting than seed limitation for recruitment in grasslands, but both were important in our experiment. The richness and abundance of native forbs increased with seed addition and the amount of increase depended on which other treatments were applied (Table S4.2 in Appendix S4.1).

Recruitment of the three sown species that were already present at the site relied entirely on seed addition, and nine of the 11 unsown native forb species recorded in our plots (Table S4.6c in Appendix S4.2) also failed to recruit. This is probably due to seed limitation as tussock thinning and litter removal had created many sites suitable for

germination and establishment (Dybzinski and Tilman, 2012; Clark *et al.*, 2007). I cannot be sure of the age of the extant forbs or when the last natural recruitment event occurred, but the unsown native forbs on our site (predominantly perennial) may not have successfully recruited for many years–perhaps since the last major disturbance event (Lauenroth and Adler, 2008) 12 years earlier.

Seed limitation is common in plant communities and often occurs in combination with a limitation of resources needed for germination and establishment (Eriksson and Ehrlén, 1992; Clark *et al.*, 2007). The arrival of seed from source populations may be affected by interactions between landscape factors (e.g. habitat and population fragmentation and isolation, pollinator availability) and species attributes (e.g. population sizes, dependence on pollinators, genetic self-compatibility/incompatibility) (Hobbs and Yates, 2003; Aguilar *et al.*, 2006; Heinken and Weber, 2013). Dispersal success is influenced by seed characteristics (e.g. size, dispersal appendages), release height (Thomson *et al.*, 2011), and landscape and site conditions (Soons *et al.*, 2005). Dispersal over time is limited by seedbank longevity, which for Australian native forb species is generally short (Morgan, 1998b). Our results suggest that seed for the sown native species and almost all the unsown existing native species were neither present in the seedbank nor dispersing to the site in sufficient quantities—probably a consequence of insufficient numbers of reproductive individuals within dispersal range (Nathan and Muller-Landau, 2000; Scott and Morgan, 2012a).

Structural influence

Structure influences the recruitment of native forbs directly through physical effects and indirectly by moderating the availability of resources (Davis *et al.*, 2000). Tussocks and litter take up space and create a physical barrier that can restrict seedling emergence (Donath and Eckstein, 2010) or prevent seeds from reaching mineral soil (Ruprecht and Szabó, 2012). Live tussock cover influences forb recruitment indirectly by competing for available soil resources and light (Loydi *et al.*, 2015; Dybzinski and Tilman, 2012). Litter reduces seedling emergence indirectly by reducing the amount of light at ground level (Foster and Gross, 1998), although accumulated leaf litter can also be beneficial for

seedling emergence in dry conditions through temperature moderation and increased water retention (Loydi *et al.*, 2013).

Exotic forb seedling abundance increased with litter removal, but I found that the abundance of sown native forb seedlings benefited relatively more than exotic species from litter removal than other treatments (based on *Delta-BIC*, Table 4.1b-c). I was unable to determine the degree to which litter depth restriction on native seedlings was related to physical obstruction or the availability of light, but exotic seedlings were not significantly restricted by litter depth. Our results suggest that exotic forb seedlings can cope with a greater litter depth. Therefore, in productive grasslands where litter accumulates, periodic removal of litter build-up is essential for maintaining the richness and abundance of native forb species as a persistent litter layer will favour the recruitment of exotic species over natives, leading to an increased proportion of exotic forbs. There were no positive forb seedling responses to litter in our study; even the abundance of established unsown native forbs was negatively associated with the litter depth existing before the experiment.

Response to resource availability

Although the richness and abundance of native forbs generally exhibited the strongest associations with structural attributes of grassland (i.e., litter depth and tussock cover), there were significant associations with some of the measured resources. For example, native forb species richness was negatively associated with soil phosphorus and positively associated with light penetrating the canopy (measured above the litter). Negative associations between elevated soil phosphorus (e.g., from the application of fertilizer or introduction of livestock) and the richness of native forbs has been widely observed (Dorrough and Scroggie, 2008; Seabloom *et al.*, 2015; Morgan *et al.*, 2016). Most native species are unable to compete with exotic species in soils with high soil phosphorus levels, as many exotic species evolved in, and are better adapted to, soils high in phosphorus (Daehler, 2003). Increased light penetrating the canopy benefits seedling and adult forbs that have grown above the litter, and it may also benefit seedlings that need light to grow through the litter (by increasing the amount of light penetrating into the litter), in this way reducing the severity of litter restriction, as per Hautier et al. (2009). The abundance of exotic forbs was more strongly associated with the amount of light penetrating the canopy

than the depth of the litter. Exotic forb species in our study are generally better adapted for rapid growth and therefore have a greater need for resources, including light (Borer *et al.*, 2014; Neuenkamp *et al.*, 2016).

While native forb seedling abundance was not associated with resource availability, most seedlings were counted while very small, and it is likely that resources would become more limiting with increasing competition among growing seedlings.

Implications for management

Experimental seed addition increased the richness and abundance of native forbs, especially when combined with tussock thinning and litter removal. Living grass tussock cover can be reduced by fire, selective herbicide application, or physically removing a proportion of individual plants. Litter build-up can also be minimised by periodic burning, or physical removal. Grazing can also reduce grass cover and litter build-up, but frequent grazing may be counter-productive as it leads to a reduction in native forbs and an increase in exotic species (Dorrough *et al.*, 2004). Care should be taken that management actions to reduce grass cover and litter build-up do not exceed thresholds required by vulnerable grassland biota. For example, Howland et al (2014) found that the species richness and abundance of ground-dwelling reptiles declined following a change in grassland structure caused by grazing. However, environmental thresholds are likely to be species-specific and may require additional research and choices of which species to favour.

The removal of exotic species was also found to benefit sown native forb abundance to some extent, even though exotic species were initially scarce at our site (Table 4.1b, Fig. 4.2b). The removal of exotic species is likely to cost less and be more effective in the long-term if populations are removed while small (Rejmánek and Pitcairn, 2001; Simberloff *et al.*, 2013). It would also be preferable to control exotic species before taking actions to reduce tussock cover or litter depth, as reduced biomass may encourage the expansion of existing exotic species.

A key result of our study was that litter restricted the abundance of emerging sown native forb seedlings more than exotic forb seedlings. The most likely reason for this is that Australian grassland species have evolved where the amount of litter was generally less

than in Europe—due to greater biomass productivity in European grass species (Groves *et al.*, 2003), and a lower likelihood of fire that removes litter (Bond *et al.*, 2005). Indeed, I demonstrated negative impacts on native forbs where the average ground litter mass was greater than 310 $g.m^{-2}$, considerably less than the 500 $g.m^{-2}$ threshold suggested by Loydi et al (2013) based on research carried out mainly in Europe and USA. *Themeda triandra* grasslands, found mainly in the southern hemisphere (Hodgkinson *et al.*, 1989), were poorly represented in the meta-analysis by Loydi et al (2013). It is reasonable to conclude that litter levels can directly influence the composition of native and exotic forbs in grassland communities, and grasslands with litter levels above the native tolerance threshold are likely to become progressively dominated by exotic forbs. Such a trend may trigger a positive feedback mechanism accelerating the decline of native forbs, due to increased exotic competition for resources and a proportionally reduced native seed supply. Strategic use of litter removal on sites dominated by native tussock grasses combined with the addition of native forbs in spring can benefit native forb richness and abundance in preference to exotic forb abundance; initially by reducing litter restriction on emerging native forbs, and subsequently through greater seed supply and competition for resources from an increased presence of native species.

Acknowledgements

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Appendix S4.1

Table S4.**1.** Experimental treatments and their descriptions. Treatments were not applied in the control plots for each treatment.

Treatment	*Description*
(a) Thin tussocks	50% of tussock plants were randomly sprayed with glyphosate (concentration 7.2 g.L), using a bottomless plastic plant pot as a spray shield. The sprayed biomass was left standing.
(b) Remove litter	All leaf litter on the ground was removed by hand.
(c) Remove exotic plants	All exotic plants were daubed with glyphosate (concentration 7.2 g.L) and left to die in situ so as not to disturb the soil. This was done four times during the 8 month experiment.
(d) Add seed	Seed for 14 native forb species from the region (Appendix 2) were mixed together and scattered by hand while sheltered from the wind. The vegetation and litter was then agitated by hand to encourage seeds to fall through to the ground. A high seeding rate (0.7g per plot = 1.2 $g.m^{-2}$, per species) was used to minimise failure from seed limitation. The 14 species were selected because they are readily available for restoration projects in south eastern Australia.

Table S4.2. Sixteen treatment/control combinations with average total native forb species richness and abundance. Percentage increase in Seeded plots is shown in brackets.

		Average total native forb:		Species richness (% increase)	Abundance (% increase)
Tussocks NOT thinned	Litter NOT removed	Exotics NOT removed	No Seed	1.5	11.0
			Seed	3.3 (122%)	18 (61%)
		Exotics removed	No Seed	1.0	3.5
			Seed	2.8 (183%)	15 (338%)
	Litter removed	Exotics NOT removed	No Seed	1.7	12.3
			Seed	4.3 (160%)	20 (64%)
		Exotics removed	No Seed	1.8	13.3
			Seed	7.0 (282%)	34 (154%)
Tussocks thinned	Litter NOT removed	Exotics NOT removed	No Seed	1.2	14.3
			Seed	3.7 (214%)	26 (78%)
		Exotics removed	No Seed	2.5	12.0
			Seed	4.3 (73%)	20 (67%)
	Litter removed	Exotics NOT removed	No Seed	1.3	12.7
			Seed	9.0 (575%)	75 (488%)
		Exotics removed	No Seed	2.7	11.7
			Seed	10.3 (288%)	113 (867%)
		All Plots	No Seed	1.7	11.4
			Seed	5.6 (228%)	40 (252%)

Table S4.3. Variables used in the analysis. Response variables (a), and explanatory variables (b).

(a) Response variables	Definition	Collection method
SRSown	Species richness of sown native forb species.	Maximum species richness from the summer and autumn surveys.
noSown	Number of sown native forbs.	Maximum seedling count from the summer and winter surveys. The maximum was used because I were interested in emergence, not survival.
noExotic	Number of exotic forb seedlings.	Counted in the autumn survey. Very few exotic seedlings had emerged at the time of the spring survey.
noOthNtv	Number of unsown native forb plants.	Counted in the autumn survey.

(b) Explanatory variables	Definition	Collection method
Litter depth	Ground litter depth (cm).	Average depth of litter on the ground for the area not covered by tussock.
%Live tussock	Percentage of area covered by living tussocks.	Average of the estimated percentage of live (green) tussock cover in each plot quarter, estimated mid-summer.
%Light penetrating canopy	Percentage of the total photosynthetically active radiation (PAR) penetrating the tussock canopy.	Measured with a one metre long LI-COR LI-191 line quantum sensor. Total PAR was measured above the canopy, and penetrating PAR was the average of two readings above the litter layer, one for each plot diagonal.
Phosphorus	Available soil phosphorus (mg/kg).	Two soil samples were taken from opposite sides of the plot in the outer 10cm, then bulked together. Available phosphorus was measured within a $NaHCO_3$ extract of the soil using a Lachat QuikChem 8500 flow injection analyser.
%Soil moisture	Percentage of soil moisture by volume.	The average of two readings by a Delta-T Theta Probe ML3 taken on opposite sides of the plot in the outer 10cm. Measured once, all on the same day, four days after rain, as an indicator of soil moisture holding capacity within each plot.
%Bare ground	Percent of area that is bare ground.	Average of the estimated percentage area of bare ground in each quarter.

Table S4.4. Overall summary statistics, for (a) all response variables, and (b) significant explanatory variables.

(a) Response variable	Min	Max	Mean (SD)	(b) Explanatory variable	Min	Max	Mean (SD)
SRSown	0	10	4.2 (3.0)	*Litter depth (cm)*	0.1	2.8	0.9 (0.9)
noSown	0	193	26.3 (40.4)	*%Live tussock cover*	0	85	33 (17)
noExotic	0	188	22.9 (34.3)	*%Light penetrating canopy*	24	94	66 (15)
noOthNtv	0	63	12.5 (12.3)	*Phosphorus (mg.kg)*	0.6	8.9	4.1 (1.4)
				%Soil moisture	5	27	13 (5)
				%Bare ground	0	56	11 (16)

Table S4.5. Mean (and standard deviation) for (a) response variables and (b) significant explanatory variables in the full data set, by treatment.

(a) Response variables	Thin-Ctrl	Tussocks thinned	Litter-Ctrl	Remove litter	Exotics-Ctrl	Remove exotics	Seed-Ctrl	Add seed
SRSown	1.6 (2.3)	2.6 (3.5)	1.0 (1.5)	3.1 (3.6)	1.9 (2.6)	2.3 (3.2)	0 (0)	4.2 (3.0)
noSown	3.8 (7.0)	22.5 (41.5)	2.4 (4.4)	24.0 (41.2)	10.2 (23.1)	16.1 (37.4)	0 (0)	26.3 (40.0)
noExotic	11.7 (17.6)	30.0 (38.7)	13.4 (20.1)	28.3 (38.2)	22.9 (33.9)	18.8 (28.6)	20.0 (33.3)	21.7 (29.4)
noOthNtv	12.1 (14.4)	12.9 (9.6)	12.5 (11.6)	12.5 (12.8)	13.3 (12.7)	11.7 (11.7)	11.4 (10.8)	13.6 (13.5)
(b) Explanatory variables								
Phosphorus (mg.kg)	4.1 (1.3)	4.1 (1.4)	4.2 (1.4)	4.0 (1.3)	4.2 (1.5)	4.0 (1.2)	4.1 (1.3)	4.1 (1.5)
%Live tussock cover	44.1 (16.0)	21.7 (9.6)	30.5 (18.2)	35.3 (16.0)	32.0 (16.7)	33.8 (17.8)	33.5 (18.1)	32.3 (16.3)
Litter depth (cm)	0.9 (0.9)	0.9 (0.8)	1.6 (0.6)	0.1 (0.1)	1.0 (0.9)	0.8 (0.8)	1.0 (0.9)	0.8 (0.8)
%Bare ground	7.6 (11.4)	14.9 (17.9)	0.5 (1.5)	22.1 (15.6)	9.4 (13.2)	13.2 (17.2)	10.6 (14.8)	11.9 (16.1)
%Soil moisture	12.6 (4.7)	13.3 (4.9)	14.6 (5.3)	11.2 (3.4)	12.0 (4.4)	13.8 (4.9)	13.5 (4.9)	12.4 (4.6)
%Light penetrating canopy	58.8 (13.3)	73.7 (11.7)	60.8 (14.4)	71.7 (12.6)	67.6 (12.7)	64.9 (16.1)	64.0 (16.2)	68.6 (12.3)

Appendix S4.2

Table S4.6. (a) Sown native species. Name, family, life form, life cycle, seeds per gram, and maximum germination (* = already present in low numbers, ** = not present at the site but present in the nature reserve, *** = not previously found in the reserve). (b) Unsown native species. Name, family, life form, life cycle. (c) Unsown exotic species. Name life cycle, and species origin.

Species		Family	Life form	Life cycle	Est. seeds.g	Max germ
(a) Sown native forbs						
Bulbine bulbosa	*	Asphodelaceae	Geophyte	Perennial	528	2.4%
Calocephalus citreus	***	Asteraceae	Chamaephyte	Perennial	10,387	-
Chrysocephalum apiculatum	**	Asteraceae	Hemicryptophyte-Partial	Perennial	6,750	0.3%
Chrysocephalum semipapposum	**	Asteraceae	Hemicryptophyte-Proto	Perennial	4,889	0.5%
Craspedia variabilis	**	Asteraceae	Hemicryptophyte-Partial	Perennial	2,833	0.9%
Cullen tenax	***	Fabaceae	Chamaephyte	Perennial	156	-
Dichopogon fimbriatus	**	Anthericaceae	Geophyte	Perennial	465	-
Eryngium ovinum	*	Apiaceae	Hemicryptophyte-Proto	Perennial	306	-
Glycine tabacina	**	Fabaceae	Hemicryptophyte-Proto	Perennial	156	-
Leuchochrysum albicans	***	Asteraceae	Hemicryptophyte-Erect	Perennial	1,316	1.4%
Linum marginale	***	Linaceae	Hemicryptophyte-Proto	Perennial	826	3.8%
Plantago varia	*	Plantaginaceae	Hemicryptophyte-Erect	Perennial	461	5.9%
Vittadinea muelleri	**	Asteraceae	Hemicryptophyte-Proto	Perennial	943	10.0%
Wahlenbergia stricta	**	Campanulaceae	Hemicryptophyte-Proto	Perennial	18,500	0.2%
(b) Unsown native species previously existing at the site						
Bulbine bulbosa		Asphodelaceae	Geophyte	Perennial		
Cheilanthes austrotenuifolia		Pteridaceae	Hemicryptophyte-Proto	Perennial		
Cymbonotus preissianus		Asteraceae	Hemicryptophyte-Flat	Perennial		
Eryngium ovinum		Apiaceae	Hemicryptophyte-Proto	Perennial		
Eucalyptus spp		Myrtaceae	Phanerophyte	Perennial		
Geranium solanderi		Geraniaceae	Hemicryptophyte-Proto	Perennial		

Hydrocotyle laxiflora	Apiaceae	Hemicryptophyte-Erect	Perennial
Leptorhynchos squamatus	Asteraceae	Hemicryptophyte-Partial	Perennial
Lomandra filiformis	Lomandraceae	Hemicryptophyte-Erect	Perennial
Plantago varia	Plantaginaceae	Hemicryptophyte-Erect	Perennial
Triptilodiscus pygmaeus	Asteraceae	Therophyte	Annual

(c) Unsown exotic species	Life cycle	Origin (http://plantnet.rgbsyd.nsw.gov.au, accessed 10/1/2016)
Conyza bonariensis	Annual	South America
Centaurium tenuiflorum	Annual	Europe
Cirsium vulgare	Biennial	North Africa, Europe, Asia
Galium divaricatum	Annual	Europe to south east Asia
Hypochaeris glabra	Annual	Europe, Asia, Africa
Hypochaeris radicata	Perennial	Europe
Hypericum perforatum	Perennial	Asia, Europe, Africa
Lactuca serriola	Biennial	Europe, Asia
Linaria pelisseriana	Annual	South Europe
Myosotis discolor	Annual / Biennial	Europe
Pertrorhagia nanteuilii	Annual	Europe
Plantago lanceolata	Annual / Biennial	Europe, north and central Asia
Tolpis barbata	Annual	Mediterranean
Tragopogon dubius	Biennial	Europe
Trifolium spp	Annual	Europe, Mediterranean, Middle East, north Africa, west Asia

Chapter Five:

Restoring and maintaining forb diversity in natural temperate grassland

In the final chapter I draw on the results of the previous chapters suggesting that soil phosphorus and biomass cover are the main threats to native forbs in productive temperate grasslands. I use thresholds for these variables, identified in Chapter four, and other research to propose a conceptual model of native forb habitat suitability based on existing phosphorus and biomass levels, and suggest appropriate restoration and management actions for practitioners.

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Abstract

The diversity of native forbs in grasslands has declined due to nutrient enrichment, biomass accumulation and invasion by exotic species. Native forb species in natural temperate grasslands frequently persist as small, isolated populations which may not produce enough viable seed, or are too far from other populations, to be self-sustaining. Thus, re-introducing native forbs and expanding the size and reducing fragmentation among existing populations are common aims of grassland conservation. Here I present a conceptual model that captures the key threats to native forbs in temperate grasslands in south-eastern Australia: soil phosphorus enrichment, biomass cover and population sizes too small and fragmented to be self-sustaining. I propose indicative thresholds for these variables as guidelines for assessing the suitability of habitat for native forbs and identifying appropriate management options for grasslands in different condition states.

Keywords: Biomass; Box-gum woodland; Exotic plant invasion; Grassland management; Grassland restoration; Grassy-box woodland; Leaf litter; Native forb; Soil phosphorus; Temperate grassland;

Introduction

Natural grasslands are threatened by agriculture, urbanisation and altered disturbance regimes (McDougall and Morgan, 2005; Öster *et al.*, 2009; Tremont and Mcintyre, 1994; Brandt and Seabloom, 2012). These threats have led to widespread declines in the diversity of broad-leaved herbs (forbs) (Tremont and Mcintyre, 1994; McCain *et al.*, 2010; Pallett *et al.*, 2016). Reductions in forb diversity can, in turn, lead to reduced protection from soil erosion, water filtration, temporal stability in ecosystem productivity and aesthetic values; and greater exposure to exotic plant invasion (Tscharntke *et al.*, 2005; Tilman *et al.*, 2006; Wratten *et al.*, 2012). Native forbs also provide habitat and resources for other species, including those that perform ecosystem services such as pollination and biological pest control (Schmidt-Entling and Döbeli, 2009; Williams *et al.*, 2015). Restoring and

maintaining forb diversity is thus a key conservation goal in grasslands around the world (Hobbs *et al.*, 2013; Öster *et al.*, 2009; McIntyre, 2011).

In Australia, Natural Temperate Grasslands are listed as Critically Endangered in the *Environment Protection and Biodiversity Conservation Act 1999*. Less than 0.5% of the original extent of intact lowland grassland ecosystems remains in south-eastern Australia, highlighting the urgent need to protect the remaining grasslands and restore diversity to modified grasslands (Prober *et al.*, 2013; Tremont and Mcintyre, 1994). Like elsewhere in the world, the loss of native forbs from Australian grasslands is often associated with enhanced soil fertility, competition from exotic plant species, and excess biomass accumulating due to modified disturbance regimes (Daehler, 2003; Hobbs and Huenneke, 1992). Despite over three decades of research trying to understand these threats (Dorrough and Scroggie, 2008; McIntyre and Lavorel, 2007; Prober *et al.*, 2013; Tremont and Mcintyre, 1994; Lunt, 1997a), successful restorations remain difficult to achieve (Morgan and Williams, 2015; McIntyre *et al.*, 2017; Gibson-Roy *et al.*, 2010). Further, actions designed to increase the richness and abundance native forbs may inadvertently inhibit native forbs by facilitating the exotic species they compete with (Faithfull *et al.*, 2012; Driscoll and Scheiner, 2017). A better understanding of differences between responses of native and exotic forbs to key environmental variables may help to protect or restore populations of native species that are self-sustaining (Prober and Wiehl, 2011; Tognetti and Chaneton, 2015; Driscoll and Scheiner, 2017).

In addition to reducing or totally removing native forb populations through grazing, pasture improvement and cropping, agriculture may leave a legacy of increased soil fertility and changed vegetation structure and soil surface condition (Dorrough and Scroggie, 2008; McIntyre and Tongway, 2005). Self-sustaining forb populations, which do not rely on ongoing management to persist, are most likely occur in areas where the land has not been cultivated or exposed to heavy grazing by domestic livestock and natural disturbance regimes (e.g. fire) have been maintained (McIntyre and Lavorel, 1994; Prober *et al.*, 2007). However, remaining intact diverse grasslands exist mostly as small isolated remnants, some of which may not produce, or receive via seed rain or the seed bank, enough viable seed or propagules to enable populations to be self-sustaining (Clark *et al.*, 2007; Tremont and

Mcintyre, 1994; Prober *et al.*, 2013). Effective dispersal of propagules between grassland remnants depends on characteristics of the surrounding landscape (e.g. habitat and population fragmentation and isolation, pollinator availability) and attributes of the target species (e.g. population sizes, dependence on pollinators, seed size, seed appendages, genetic self-compatibility/incompatibility) (Hobbs and Yates, 2003; Aguilar *et al.*, 2006; Heinken and Weber, 2013).

This paper is a synthesis of current research on the threats facing native forbs and how to manage grassland diversity. I build on McIntyre and Lavorel's (1994) conceptual synthesis of human-induced disturbance as a key factor shaping the composition of native and exotic species in contemporary grasslands. I present a conceptual model that illustrates typical grassland states resulting from native plant community responses to changed soil nutrients and accumulated biomass (phytomass, living and dead plant material) produced by grasses (including C3, C4, annual and perennial species) (Fig. 5.1). While our model is based on surveys carried out in late spring/summer only, and therefore may not capture all grass species within their growing season, I believe that the role living and dead grass biomass in this model are the same, regardless of phenology. The model is intended to help practitioners predict a site's potential to support native forbs and devise strategies for increasing the diversity of native forbs.

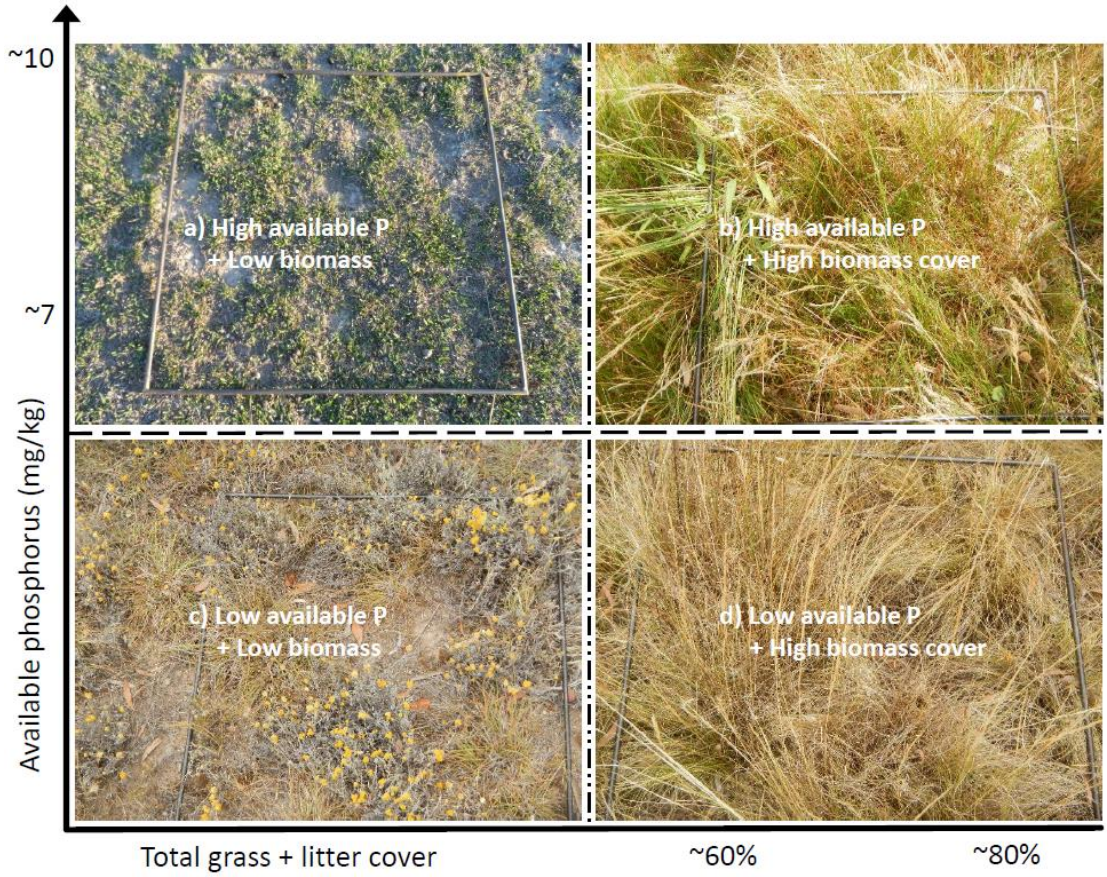

Fig. 5.1. Typical grassland states where a) soil phosphorus is high, biomass cover is low; b) soil phosphorus is high, biomass cover is high; c) soil phosphorus is low, biomass cover is low; and d) soil phosphorus is low, biomass cover is high. (Photos by D. Johnson)

A conceptual model of habitat suitability for native forbs

I propose a model based on a review of empirical research conducted in Australian grasslands (McIntyre, 2010; Wong *et al.*, 2010; Dorrough *et al.*, 2011; Morgan, 1998c; Schultz *et al.*, 2011), including our own study, in which I identified limits in the ranges of available phosphorus, grass and litter cover within which native forbs are likely to occur (Johnson *et al.*, Chapter 2). I suggest four zones of habitat suitability for grassland native forbs based on approximate thresholds of available phosphorus in the soil, biomass and availability of seed (Fig. 5.2a-d). The zone with low available phosphorus and low biomass

(Fig. 5.2c) represents conditions likely to exist in relatively unmodified natural grasslands that provide suitable habitat for native forbs. In contrast, native forbs are unlikely to reproduce or compete with exotic species effectively in grasslands represented by the zones with high available soil phosphorus and/or high biomass (Fig. 5.2a, b, d).

While plant growth requires macronutrients, including nitrogen and phosphorus and seven others, biodiverse communities require either phosphorus or nitrogen limitation to prevent dominant species from excluding all other species (Schelfhout *et al.*, 2015). Therefore, phosphorus and nitrogen levels are often associated with plant diversity, sometimes depending on concentration ratios with other elements such as carbon (Driscoll *et al.*, 2018), but biodiversity is usually highest under phosphorus limitation (Schelfhout *et al.*, 2015). I use available phosphorus in our model as phosphorus levels are more stable than nitrogen because phosphorus binds tightly to soil particles and persists much longer in soils; nitrogen is more mobile within soils and fluctuates due to plant decomposition, nitrogen-fixing and leaching (Ceulemans *et al.*, 2011). Rather than measuring biomass by weight (e.g. g/m^{-2}) (Schultz *et al.*, 2011; Loydi *et al.*, 2013), I measured biomass as living grass cover plus dead litter cover (on the ground or still standing). Cover is relatively easy to measure and is related to gap size, which affects native forbs (Morgan, 1998c). Areas where biomass and soil phosphorus are below the self-sustaining thresholds may provide suitable habitat for native forbs, but the presence or absence of persistent populations of native and/or exotic forbs in those areas also depends on land-use history. Where biomass and/or soil phosphorus levels are higher than the thresholds it is likely that a greater proportion of forbs will be exotic species.

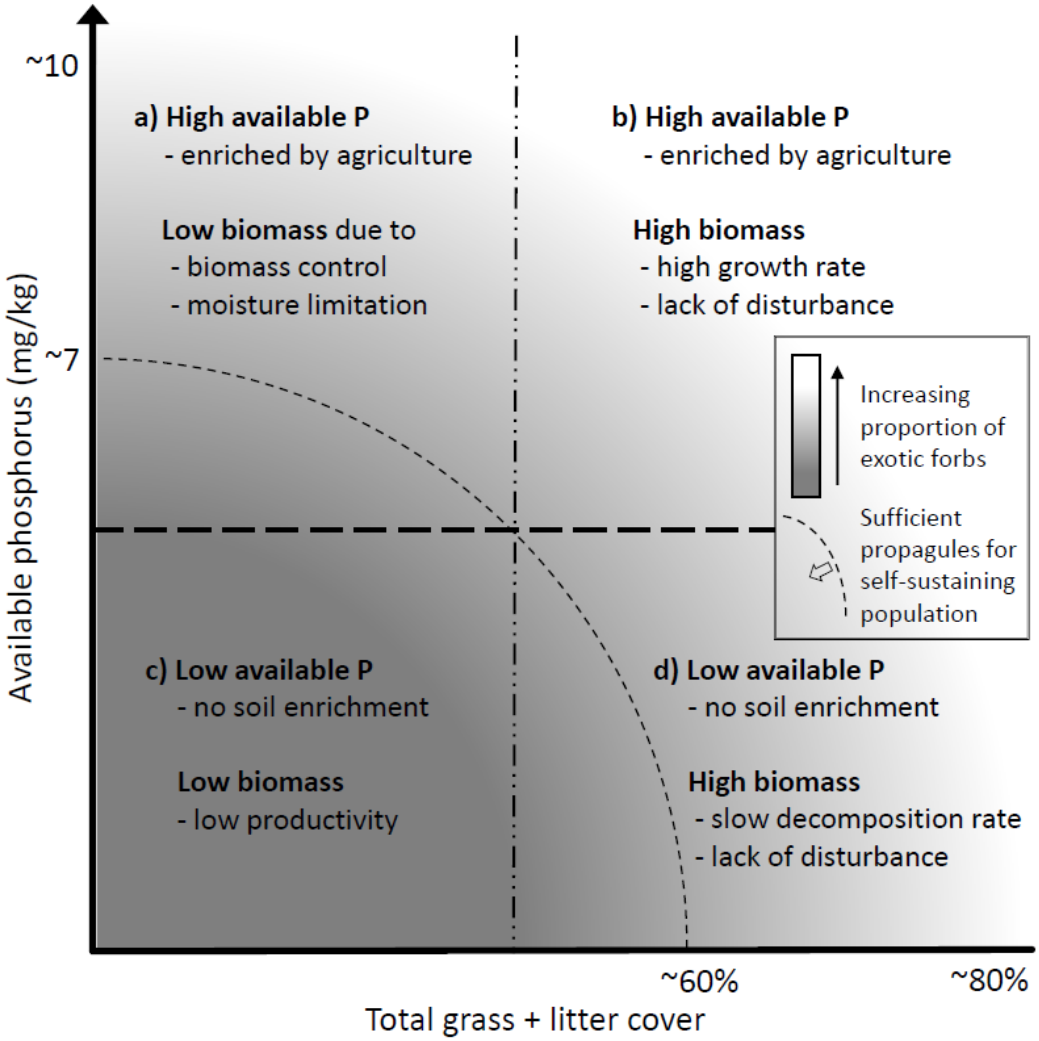

Fig. 5.2. A conceptual model of the suitability of habitat for native forbs (darker indicates higher suitability for native forbs) in relation to thresholds of available phosphorus, biomass and the availability of propagules. In zone a) native forbs are likely to be displaced by exotic forbs, as abundant resources fuel exotic competitive superiority (low biomass here may have resulted from lack of moisture, intense grazing or burning), in zone b) biomass-tolerant exotic forbs are likely to displace native forbs, in zone c) self-sustaining populations of native and exotic forbs are likely (depending on land-use history), and in zone d) native forbs are likely to be replaced by exotic forbs after the native species decline as a result of excessive biomass. Poor habitat suitability will result in native forb populations declining to a point (the dotted line, the shape and position of which is likely to be species specific) where they are no longer self-sustaining.

Native forbs need low grass and litter cover

Total grass and litter cover in Fig. 5.2 (hereafter, biomass cover) is the % cover of living grasses and dead litter (on the ground or still standing) and can be estimated by visual assessment. Biomass cover affects the regeneration niche of native forbs by reducing light availability and it may physically obstruct emerging seedlings (Loydi *et al.*, 2013; Johnson *et al.*, 2018). Exotic forbs are more tolerant of high levels of biomass than native forbs because they evolved in grasslands with greater biomass productivity and lower frequency of fire (Groves *et al.*, 2003). Living grasses compete with forbs of all ages for light, nutrients, and moisture (Staples *et al.*, 2016; Loydi *et al.*, 2015). Johnson et al (Chapter 2) found almost zero probability of a native forb species occurring where combined litter and grass cover was over 80%, (with the larger fraction being grass cover). Notably, native forbs tolerate higher levels of grass cover than litter cover (Johnson *et al.*, 2018). Other research has identified similar high levels of mortality among emerging native forb seedlings where the cover of dominant grasses is high (Morgan, 1998c; Schultz *et al.*, 2011). I propose an indicative threshold of 60% for grass and litter cover combined (e.g. up to 25% litter + 35% grass)(Chapter 2) for self-sustaining populations.

Low soil fertility helps native forbs compete with exotic species

Increasing levels of available soil phosphorus are widely associated with reduced plant diversity and increasing proportions of exotic species in grasslands (Seabloom *et al.*, 2015). Abundant nutrients allow species with greater competitive ability (i.e., many exotic species) to grow rapidly and deprive less competitive species, including native forbs, of light (Hautier *et al.*, 2009). Typically, native forbs are unlikely to persist where available phosphorus exceeds 10 $mg.kg^{-1}$ and I suggest a threshold of approximately 7 $mg.kg^{-1}$ (Fig. 5.2) for self-sustaining populations (Johnson *et al.*, Chapter 2). Appropriate thresholds for forb responses to phosphorus may vary by location due to interactions and adaptations to different climates, soil types, and land-use history. For example, one study in the ACT

identified a range of 1-6 mg/kg^{-1} (McIntyre, 2010) and two studies in Victoria identify slightly higher levels of 8 mg/kg^{-1} (Dorrough *et al.*, 2011) and 10 mg/kg^{-1} (Wong *et al.*, 2010). Available phosphorus can be measured using various laboratory techniques, for example that measure phosphorus concentration in $NaHCO_3$ solution (Colwell, 1963).

As an alternative to expensive soil analyses, practitioners may be able to estimate whether soil phosphorus levels are high or low by interpreting cues from the traits of the dominant existing plant species. A high proportion of species with plant traits associated with slow growth, such as small thick leaves (low specific leaf area, high leaf-dry-matter-content) and high stem-tissue density, suggests low nutrient availability in that location, as opposed to large thin leaves which suggest high nutrient availability (Jager *et al.*, 2015; Shipley *et al.*, 2017). Traits associated with slow growth are common among Australian native forb species, and a relatively high proportion of native vs exotic forbs (species richness or cover) indicates low soil fertility (Dorrough *et al.*, 2006; Johnson *et al.*, Chapter 2). However, I cannot say that a lack of native forbs indicates high fertility as they may have been lost due to previous land-use (e.g. over-grazing) (Morgan, 1998b; McIntyre and Lavorel, 2007). Nor can I say that a higher proportion of exotic species indicates high fertility as some exotic species are also well-adapted to low soil fertility (Johnson *et al.*, Chapter 2; Driscoll and Scheiner, 2017).

The occurrence of individual species may also provide an indication of fertility (Karltun *et al.*, 2013). For example, species indicating low soil phosphorus include the native grass, *Aristida ramosa*, and the native forb, *Chrysocephalum apiculatum*. Species indicating high phosphorus include the exotic grass, *Phalaris aquatica*, and the exotic forb, *Acetosella vulgaris* (McIntyre, 2010; Johnson *et al.*, Chapter 2). However, some species (e.g. *Microlaena stipoides*) are poor indicators of soil fertility due to considerable variation in their growth relationships with soil phosphorus (McIntyre, 2010).

Self-sustaining populations

It is not easy to tell if existing populations of native forbs are self-sustaining. Most native forbs are visible for limited periods during autumn and/or spring if seasonal conditions are suitable. They may not be visible at other times, or if conditions are harsh, but they may be

living in a dormant state underground, an adaptation that allows them to survive long periods of drought (Lunt, 1997b; Morgan, 1998b; Scott and Morgan, 2012b). Most native forbs are perennial species that rely on long-lived individuals to remain present over many years and thus it may be difficult to determine whether a population is self-sustaining as long life-spans can conceal population decline, which may not become evident for many years (i.e. extinction debt) (Brandt and Seabloom, 2012). For populations to persist in the long-term they need to recruit from seed. Populations depend on successful recruitment of new seedlings when conditions are favourable, as seeds or seedlings may fail if conditions are poor, and un-germinated seed for native forb species does not stay viable for long in the soil (Morgan and Williams, 2015). Likewise, the population size and density needed to be self-sustaining depends on the species (Fig. 5.2), some of which are naturally rare and widely dispersed. The appearance of many new seedlings in favourable seasons would indicate that those species are recruiting successfully. However, long-term monitoring will give the best indication of whether the recruitment rate is sustaining a population.

Management/restoration options for grasslands

The location of a site with respect to thresholds of available phosphorous, biomass cover and the availability of propagules (Fig. 5.2), provides cues to restoration options for grassy ecosystems (Fig. 5.3). Here I discuss restoration options where available phosphorus is low (Fig. 5.3c,d), and where high levels of available phosphorus exist (probably a legacy of agricultural land-use) (Fig. 5.3a,b), with or without high biomass cover. Restoration options for the latter are likely to be more radical and more costly.

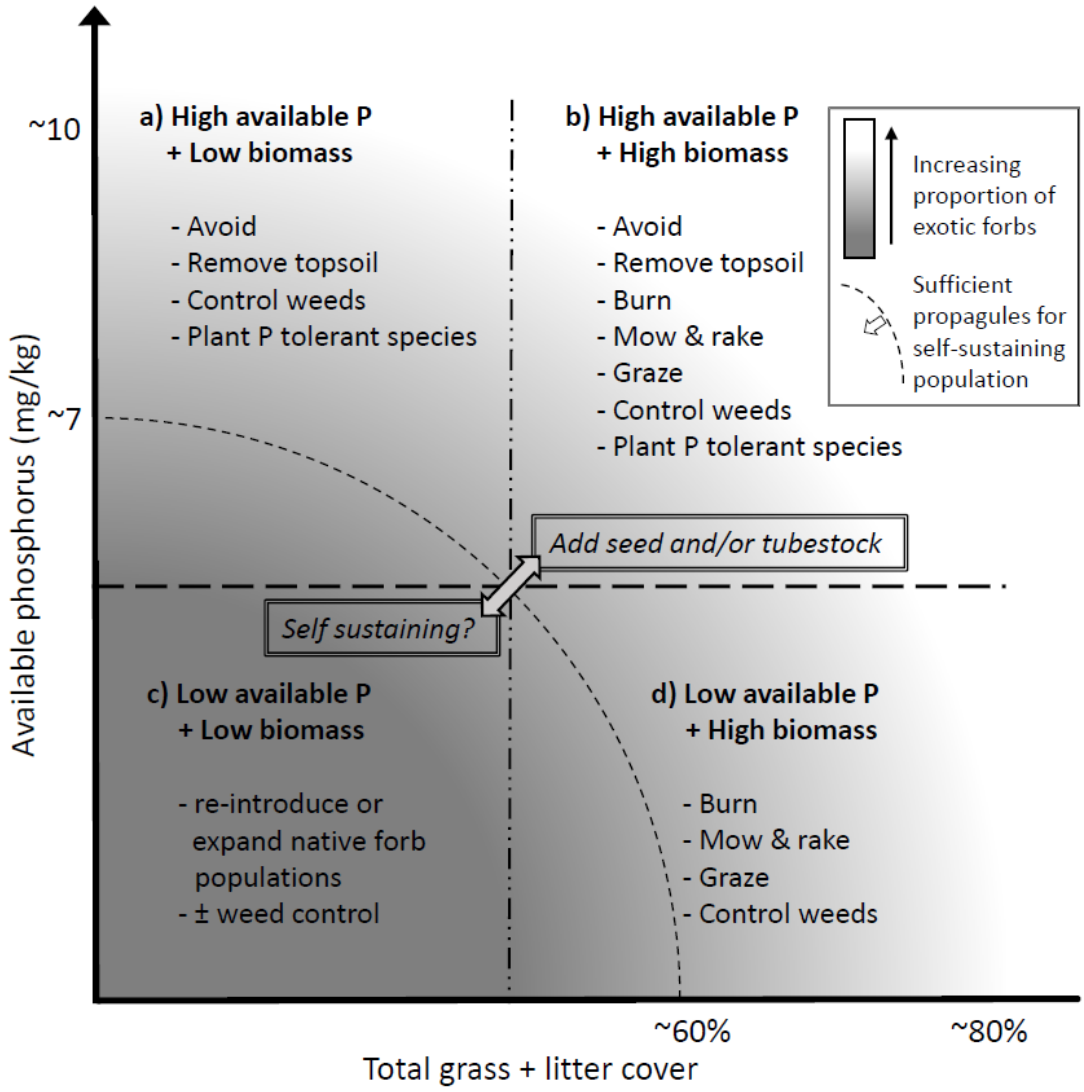

Fig. 5.3. Recommended actions for restoration in relation to thresholds of available phosphorus, biomass and propagule availability. In zone a) successful native forb restoration may require topsoil removal, which removes most of the phosphorus enrichment and exotic seedbank, although there will be a period when bare soil is exposed to erosion and reinvasion by exotic plants. Natural depletion of soil phosphorus occurs very slowly (over decades) and trialed methods for increasing the depletion rate have had limited success (Schelfhout *et al.*, 2015; Cole *et al.*, 2016). In zone b) biomass control may improve conditions for restoration if topsoil removal is not feasible, but the on-going risk of intense competition from weeds would be difficult and costly to manage. In zone c) self-sustaining populations of native forbs (depending on land-use history) may persist with

little intervention. Biomass management in adjoining areas, and reducing the cover of exotic species, may encourage populations to grow and expand. In zone d) biomass control combined with the control of exotic species (mainly forbs and annual grasses) should create conditions for successful native forb restoration. Seed or seedlings of native species may need to be added where existing populations are not large enough (depending on the species) to be self-sustaining.

Managing forbs in areas with low P and low biomass

Low soil phosphorus and low biomass (Fig. 5.3c) provide habitat conditions that allow native and exotic forbs to co-exist. Domination by native forbs under these conditions may suggest that no restoration is required, and effective management may simply entail on-going monitoring to assess whether recruitment rates are falling, or exotic cover is increasing. Exotic forbs and grasses are unlikely to threaten stable native forb populations (depending on the native and exotic species present) but reducing exotic cover may be beneficial even where phosphorus levels are low. Exotic plants (mainly forbs and annual grasses) compete with native forbs for resources, and their removal may improve recruitment and facilitate population expansion of native species (Johnson *et al.*, 2018). The choice of weeding method should take the likely benefit to native forbs and risks of impacts on other non-target species into account as well as practical considerations such as cost (Flory and Clay, 2009).

If native forb populations are small or absent during ‘good’ seasons in areas with low phosphorus and low biomass, it is unlikely that sufficient propagules are present, and restoration will require the importation of forbs as seed or seedlings (e.g. tubestock) (Johnson *et al.*, 2018). The use of fire is not recommended in areas where biomass levels are low due to low productivity; because there is little benefit to be gained, and further research is needed to understand how some grass species are affected by fire, and at which time of year burning is most beneficial (Morgan, 2015). Burning areas of mixed high and low productivity could require additional care to protect low productivity patches (e.g. by pre-wetting).

Restoring forbs in areas with low P and high biomass

Few native forb species are likely to persist in high biomass (Fig. 5.3b,d), although if phosphorus is low, species which reproduce vegetatively and do not rely on seed for reproduction (as litter inhibits the seedling emergence phase) may persist longer. For example, I found that the clonal species *Hydrocotyle laxiflora* persisted in areas with high litter cover (Johnson *et al.*, Chapter 2). However, exotic forbs are less sensitive to biomass (Johnson *et al.*, 2018) and some species may persist in areas with high biomass. If exotic forbs are dominant where phosphorus is low and biomass levels are high (Fig. 5.3d), it is likely that exotic forbs 'replaced' native forbs due to the latter's intolerance of high biomass (MacDougall and Turkington, 2005; Johnson *et al.*, 2018). Provided sufficient propagules are present, native forbs may recover where there is low phosphorus if actions are taken to remove excess biomass (eg. burn, mow and rake, graze) (Morgan, 2015). Although, depending on the species present, reducing biomass may also benefit exotic forbs (Smallbone *et al.*, 2007; Catford *et al.*, 2012; Driscoll and Scheiner, 2017), and on-going management of potential exotic re-invasion may therefore be required (Kettenring and Adams, 2011; Flory and Clay, 2009).

Burning target exotic species when they are about to set seed (e.g. exotic annual grasses in spring) may reduce the risk of exotic re-invasion, but the timing of burns may be limited by fuel loads or other management considerations (Prober *et al.*, 2009). Some native forbs may recover from underground tubers if present if the soil has not been disturbed by farm machinery (e.g. ploughed) (Morgan and Williams, 2015), but it will probably be necessary to add seed or seedlings since native forbs have a short-lived seedbank (Morgan, 1998b). New seedlings may also benefit from the removal of competition from exotic species (Kettenring and Adams, 2011; Johnson *et al.*, 2018).

Restoring forbs in areas with high P and high biomass

Exotic species are likely to dominate where there is high phosphorus and high biomass (Fig. 5.3b), although several native species may persist in areas with medium phosphorus levels (e.g. *Oxalis perennans*) (Dorrough *et al.*, 2011). Reducing biomass (eg. by burning, mowing, grazing) to levels tolerated by native forbs, followed by the addition of native forb

seed or seedlings, the removal of exotic species (to reduce exotic competition and potential exotic seed sources at landscape level), and on-going weeding to prevent re-invasion, may increase the presence of native species temporarily. However, the necessary weeding costs would be high, and the risk of native forb species failing to establish will also be high (Bauer, 2012; Kettenring and Adams, 2011).

Removing a layer of top-soil before sowing seed and/or planting may improve the likelihood of successful restoration by removing much of the excess phosphorus, and the exotic seedbank, allowing native forbs to re-establish without high levels of competition from exotic species (Gibson-Roy *et al.*, 2010). However, this is only recommended in flat areas with sufficient topsoil depth (10-20 cm) and few rocks. This treatment also leaves bare ground unprotected from erosion and re-invasion by exotics for a long period of time and is only cost-effective on a small scale (Ceulemans *et al.*, 2011; Schelfhout *et al.*, 2017). The estimated cost of removing topsoil and establishing native forbs and grasses is AUD$40,000 per hectare (Greening Australia) (Richard Milner, ACT Government; pers. comm.). Non-conclusive trials of alternate methods of depleting soil phosphorus levels (Cole *et al.*, 2016; Schelfhout *et al.*, 2015) suggest that the best strategy for increasing forb diversity on a larger scale may be to avoid phosphorus-enriched areas (Ceulemans *et al.*, 2013; Schelfhout *et al.*, 2017).

Restoring forbs in areas with high P and low biomass

High phosphorus and low biomass levels (Fig. 5.3a) may occur where plant growth is inhibited (e.g., by moisture shortage during drought) or biomass has recently been removed (e.g., by heavy grazing or burning). If available water is inhibiting exotic forbs then restoration is probably not needed (or possible), otherwise restoration approaches are the same as described for high phosphorus and high biomass.

Adding seed or seedlings to boost re-establishment of native forb populations

Restoration may rely on adding seed or planting seedlings if existing native forb populations are too small to produce enough seed to be self-sustaining and there are insufficient numbers of reproductive individuals in other populations within dispersal

range, (Nathan and Muller-Landau, 2000; Scott and Morgan, 2012a). Biomass and weed control may be necessary prior to seed addition as seedling emergence among native forbs is sensitive to litter and competition from exotic species (Johnson *et al.*, 2018). High seeding rates (0.5- 1.0 g/m^{-2} of mixed species) are recommended as germinated seedlings of many native species have a high mortality rate (Zamin *et al.*, 2018; Morgan and Williams, 2015); although lower rates may be adequate for species with a high recruitment success rate (Jaksetic *et al.*, 2018).

In areas where biomass levels are higher, seedlings transplanted as tube-stock may have greater success than seedlings grown from sown seed, as established seedlings may avoid the restriction of litter on seedling emergence and be less sensitive to competition with exotic species (Morgan, 1998c). Propagule pressure from exotic species (within and surrounding these sites) is often likely be greater than the propagule pressure from native species (Catford *et al.*, 2012). Tube-stock planting on a large scale can be made easier with planting tools such as the Hamilton planter (www.forestrytools.com.au/index.php?id=153). Establishment success of seed scattered on the ground may improve if raked into the soil (Zamin *et al.*, 2018), but additional weed control may be required as soil disturbance is likely to facilitate the germination of exotic species more than native species (McIntyre and Lavorel, 1994).

The addition of seed or planting seedlings are increasingly more difficult and costly on a larger scale and rely on the availability of sufficient quality seed or seedlings of appropriate species, which may be limited in some regions (Gibson-Roy *et al.*, 2010). Species choice and availability may be further complicated by climate change, prompting trial restorations of species sourced from areas where the current climatic conditions are similar to predicted future conditions at the restoration site (Richard Milner, ACT Government; pers. comm.). A combination of re-introduction strategies may provide the greatest success, using species with mixed provenance, restored as seedlings where the biomass is slightly higher and from sown seed where biomass has been cleared. Where the area is too large to afford (or risk) restoring the whole area at once, a more feasible approach might be to restore small populations in a number of segregated zones, with the intention that, with on-going biomass and weed control the populations eventually become

self-sustaining and expand until they meet each other. This approach is being trialled in the ACT (Richard Milner, ACT Government; pers. comm.).

Conclusions

Our conceptual model provides a simple framework for understanding associations between soil phosphorus, biomass cover and propagules and management of habitat for native and exotic forbs. Suitable habitat conditions for native forbs may be maintained or restored relatively easily where available phosphorus levels remain low, with appropriate management to prevent excessive biomass cover in conjunction with seed/seedling addition where necessary. For ex-agricultural soils with high levels of available phosphorus, management and restoration options are fewer, more expensive and expose soils to risk of erosion. The most realistic approach under these circumstances may be to avoid those areas for restoration until after phosphorus levels have depleted to natural levels (some decades in the future), restore these sites with seedlings of desirable P tolerant species, which may include trees and shrubs (depending on your goals), or wait until alternative methods of depletion are devised (Ceulemans *et al.*, 2011). Because considerable uncertainty exists around much of the advice provided here, managers are encouraged to apply our conceptual model within an adaptive management framework. This strategy should eventually lead to better restoration options for native forbs in grassy ecosystems.

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Appendices

Appendix One

This paper is not included as part my PhD thesis. It is provided as a record of additional work produced during my candidature.

I wrote this paper in 2015 with Philip Gibbons to provide my sponsors, the ACT Government, an early return on the investment they made towards my PhD, which was some way off completion at that time. They used it to guide a broad-scale restoration program within the study area.

Restoration options for Kama Nature Reserve

Authors: David Johnson and Philip Gibbons

Fenner School of Environment and Society, The Australian National University
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Background

The following nine ground-layer communities were identified in a soil and vegetation survey of Kama Nature Reserve in December 2012. They were initially determined based on the dominant grass species and overall vegetation density, but analysis of the data showed that each community has its own characteristic attributes related to plant community composition, soil fertility, and biomass configuration, among other things. Knowledge of these attribute combinations, and the findings from our previous research at Kama, have allowed us to devise targeted strategies that are likely to be successful for increasing the proportion of native species within each community, particularly native forbs.

Guiding principles and assumptions

- Most native forb and grass species don't compete well where there is medium-high soil fertility.

- A layer of litter inhibits forb recruitment from seed as germinated seedlings die before they can penetrate the litter. Many native species are affected even if the litter layer is quite thin, affecting native species richness.

- Ongoing survival of established seedlings is governed more by resource availability, affecting the abundance of established seedling and adult forbs.

- Established native forbs can compete successfully with exotic species but long-term survival of small populations is often restricted by inadequate seed production or dispersal.

- Forbs don't form a large soil seed bed, so areas deficient of native forbs are unlikely to have a residual seed bank for restoration.

- Burning may have different results in high vs low productivity grasslands. Very little research on burning low-productivity grassland has been done, and the results so far have not shown significant increases in native richness, probably because the existing grasses weren't high in biomass anyway, and in dry areas there is a risk of destroying some of the native grasses (Morgan 2015 - "Land of sweeping plains").

Recommendations by management unit

We divided the ground layer in Kama Nature Reserve into nine management units (Figure 1). These are briefly described here, along with options for maintaining or increasing the native plant species richness in each unit. The options are based on some guiding principles and assumptions, explained above, and the question of when to burn, discussed later in this document.

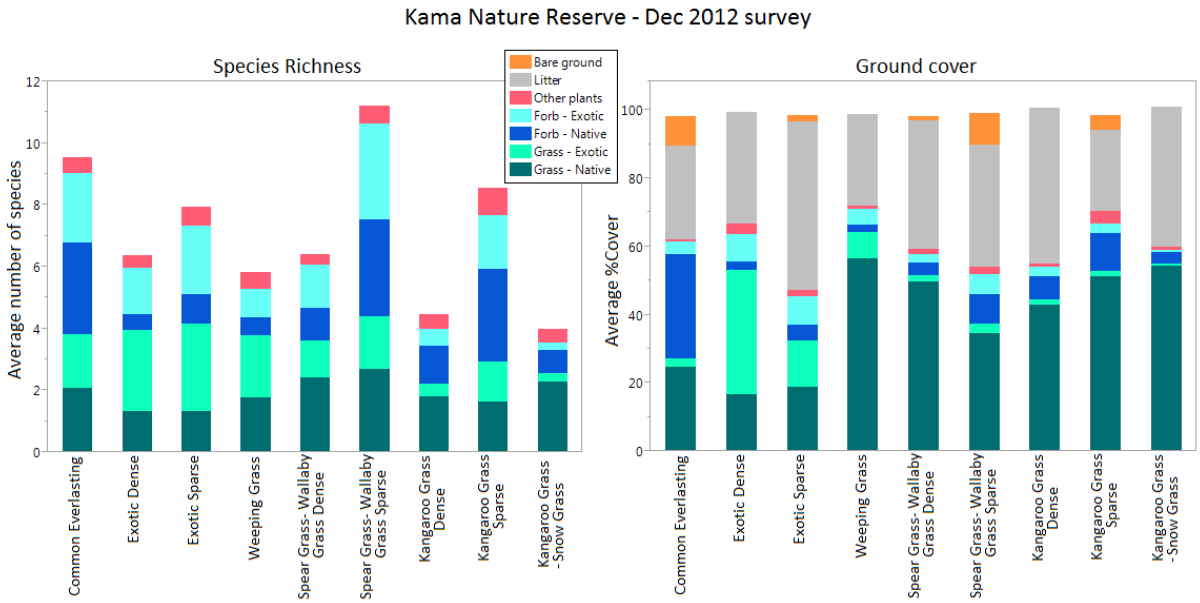

Figure 1. The ground layer of Kama Nature Reserve divided into nine management units based on species composition, structure and soil fertility.

Common Everlasting

Main grass species - *Austrostipa scabra*, *Rytidosperma*, *Themeda*. Total grass cover <30%. Dominant species is *Chrysocephalum apiculatum*.

Low soil fertility, low plant density. High diversity, roughly equal proportions of native and exotic species, for grasses and forbs. %Cover mostly native. Thin litter layer in gaps where not bare ground.

Restoration options

1. Do nothing. Already has high native forb diversity and cover, and low cover of exotic forbs and grasses. Normal ongoing seasonal/successional dynamics will continue. Low risk.

2. Reduce litter cover. This may lead to an increase in native forb cover and/or richness in areas currently covered by litter. Several ways to achieve this.
   - Manual removal may be safer, effective and feasible on smaller scales.
   - Burning may be relatively easier as scale increases, but the benefit of burning has not been proven in low-productivity grassland and care needs to be taken not to kill existing native grasses. Only a slight risk of increased exotics as the existing thin litter layer would not be providing much protection anyway.

Comment – We recommend trialing cool burns and manual removal in a few small areas before the autumn rains. Decide what to do on a broader scale later, based on the results.

Exotic Dense

Main grass species - *Avena, Bromus, Phalaris, Themeda*. Total grass cover >50%.

High soil fertility. Most grass and forb species exotic, and %cover mostly exotic. Medium litter depth in gaps. No bare ground.

Restoration options

1. Remove topsoil, sow native forb species, and then (once forbs established) plant/sow native grasses– this should lead to complete community replacement at suitable sites, but costs approximately $40,000 per ha (based on estimate by Greening Australia). Low risk of weed invasion during forb establishment if monitored and controlled – can use selective herbicide to combat grasses during this phase. Alternatively sow native grasses at the same time as native forbs (currently being trialled by Greening Australia).

2. Plant woody species to provide mid-storey and overstorey structure, reduce exotic biomass and scavenge nutrients, which may make ground-layer restoration more feasible in future.

Exotic Sparse

Main grass species - *Aira, Avena, Bromus, Briza, Themeda, Vulpia*. Total grass cover <40%.

Low-medium soil fertility. Most grass and forb species exotic. Native and exotic grass cover roughly equal. Forb %cover mostly exotic. Low-medium litter depth in gaps. Little bare ground.

Restoration options

1. Spring burn to reduce litter and interrupt exotic annual grasses setting seed, then sow warm season native grasses (eg. *Themeda triandra, Bothriochloa macra*) and forbs likely to tolerate this level of soil nutrients and low litter (eg. *Tricoryne elatior, Schoenus apogon, Plantago varia*). This should increase the native grass component and native forb abundance, and possibly richness as well under ideal conditions, depending on how much damage is inflicted on the few existing native forbs. There's a low-medium risk of exotic invasion later in spring if the sown seeds fail.

Comments – Spring burn suggested as the amount of new native forb seed present is likely to be low and the potential for an increase in exotic grasses high.

Weeping Grass

Main grass species - *Microleana stipoides, Lolium perenne*. Total grass cover >60%.

High soil fertility. More exotic grass and forb species than native. Grass cover almost completely native (*Microleana*) but forb %cover mostly exotic. Medium litter depth. No bare ground.

Restoration options

1. Do nothing. Already native grass dominated, even though it also has exotic forbs. Due to high nutrients there's a high risk of increased exotics if the litter, which is currently inhibiting further invasion, is removed.

Spear Grass-Wallaby Grass (Dense)

Main grass species - *Austrostipa bigeniculata, A. scabra, Rytidosperma, Themeda*. Total grass cover ~50%.

Medium soil fertility. More native grass species than exotic and more exotic forbs than native. Grass cover almost completely native and very low forb %cover. Medium-high litter depth. Very little bare ground.

Restoration options

1. Do nothing. Already native dominated, and due to medium nutrients there's a medium risk of exotic invasion if the litter is removed.

2. Spring burn to reduce litter and interrupt exotic annual grasses setting seed, then sow native forbs able to cope with some nutrients (*Bulbine bulbosa, Tricoryne elatior, Convolvulus erebescens, Vittadinia muelleri*). This should increase native forb presence under ideal conditions, with (assumed) reduced risk of increased exotics. If this fails, there's a medium-high risk of exotic invasion.

Comments – Spring burn suggested as the amount of new native forb seed present is likely to be low and the potential for an increase in exotic grasses high. However, there may also be a risk of fire severely damaging these native grass species (ref: current trials, Richard). Suggest trialing in small areas first.

Spear Grass-Wallaby Grass (Sparse)

Main grass species - *A. scabra, Rytidosperma, Themeda, Vulpia*. Total grass cover <40%.

Low soil fertility. More native grass species than exotic, and high exotic and native forb richness. Grass cover almost completely native, and medium forb cover and more native than exotic. Low litter depth in gaps where not bare ground.

Restoration options

1. Do nothing. Native diversity is already good and the potential benefit may not be worth the risks associated with intervention.

2. Cool autumn burn or manual litter removal, then sow native forbs. This should increase native forb presence where previously occupied by litter. Failure to establish native forbs should only result in a low risk of exotic invasion as the existing litter layer is thin.

3. Same as 2 but without sowing native forbs. Unproven diversity benefit from burning low-productivity grasslands. Relies on the presence of native forb seed from last season.

4. Totally spray out small patches (eg 1m x 1m) with non-selective herbicide (glyphosate) in spring, remove litter, and then sow with native forbs. Once the forbs are established and producing seed, manually remove litter in surrounding area to facilitate natural dispersal. This should achieve high forb richness and abundance within the patch under ideal conditions, and potentially increased native forbs in the surrounding area in the following seasons. Low risk of exotic invasion within the patch during the establishment phase if actively controlled - exotic grasses can be sprayed with selective herbicide and exotic forbs daubed with glyphosate. Low ongoing risk of exotic increase in surrounding gaps once the thin litter layer has been removed.

Comment – If willing to risk intervention, recommend trialing options 2 and 4 on small areas initially, plus 3 if resources are available, before deciding for larger areas.

Kangaroo Grass (Dense)

Main grass species – *Themeda*. Total grass cover <40%. High %cover of deep litter.

Low-medium soil fertility. Low grass richness, mostly native. More native forb species than exotic. Grass cover almost completely native, and medium forb cover, mostly native. High litter depth and no bare ground.

Restoration options

1. Spring or autumn (?) burn, then sow native forbs. High probability of increased native forb species richness and abundance under ideal conditions.

2. Where burning is not safe/allowed, a spring slash and rake may be a suitable alternative.

3. Same as 1 but with tussock thinning, using glyphosate. This should lead to an increase in the abundance of native forbs due to reduced competition, but there's a high probability that the abundance of exotics would also increase for the same

reason.

4. Do nothing, as its already native dominated and is not at risk of exotic invasion due to the thick litter layer.

Comments – Recommend trialing option 1 in both seasons (if allowed), and option 2 in spring, in small areas before deciding. Option 3 if *Themeda* density is high post-burn/slash.

Kangaroo Grass (Sparse)

Main grass species - *Themeda, Vulpia*. Total grass cover ~50%.

Low soil fertility. Low grass richness, roughly equal native and exotic. High native and medium exotic forb richness. Grass cover almost completely native, and forb cover mostly native. Relatively low litter cover and depth and some bare ground.

Restoration options

1. Do nothing. Native diversity is already good and the potential benefit may not be worth the risks associated with intervention.

2. Cool autumn burn or manual litter removal, then immediately sow native forbs. Under ideal conditions this should increase native forb presence where previously occupied by litter, and resist exotic invasion. Failure to establish native forbs would only slightly raise the risk of exotic invasion in spring as soil fertility is low and the existing litter is thin.

Comment – Recommend trialling option 2 before deciding on either for larger areas.

Kangaroo Grass-Snow Grass

Main grass species - *Poa sieberiana, Themeda triandra*. Total grass cover >50%.

Low-medium soil fertility. Low grass richness, mostly native. Low forb richness, mostly native. Grass cover almost completely native. Low forb cover, mostly native. High litter depth and no bare ground.

Restoration options

1. Do nothing, as its already native dominated and is not at risk of exotic invasion due to the thick litter layer.

2. Spring burn (or slash and rake) to reduce litter, then sow native forbs. High probability of increased native forb species richness and abundance under ideal

conditions. There may be a high risk of exotic invasion if the native forbs fail to establish well enough to compete for resources.

Comments – Spring burn is recommended here on the assumption that the low native forb diversity and cover, plus deep litter, means there won't be a good natural supply of native forb seed for germination in autumn. Also protects from exotic invasion until native forbs sown in spring.

When to burn

Most native forb species germinate readily, are not inhibited by darkness (eg. under litter) – although 2 common exceptions are *Vittadinia muelleri* and *Chrysocephalum apiculatum* - and don't have an extended dormancy. Most are likely to germinate almost entirely in autumn following the seasonally predictable 'autumn break', (from a study of 28 species by Morgan, 1998). Therefore burning to maximise germination of native seed produced last spring/summer should be done before the autumn rains, as little of this seed will remain viable until spring.

However, this advantage is reduced where there is only a low likelihood of a good supply or diversity of native forb seed, for some reason – eg. native forb cover or diversity in the area is low. In these instances we would probably be aiming to achieve increased forb diversity from additional native forb seed sowed following the burn.

The argument for burning in spring applies mainly to areas where there are exotic annual grasses present, especially if soil fertility is slightly higher. It's been suggested that an early spring burn may prevent exotic grasses from setting seed, while being cool enough and early enough not to harm native forbs significantly. Greening Australia (Nicki Taws and Suzanne Prober) are currently trialing spring (August) burns and should have some results on this in a month or so.

Where there is neither a good supply of natural forb seed, nor an abundance of exotic annual grasses nearby, then the reason for burning in one season or the other becomes less clear. In these areas the depth of the litter layer becomes a consideration, as we've found that exotic forbs are less inhibited by a thin litter layer than native forbs, although this depends on the species. Where the litter is thin it is not currently providing much protection against exotics anyway, and should be burned or removed in autumn to give any existing native forb seed the best chance of germinating, along with any additional seed you may be adding. Conversely, where the litter is deep it is preventing exotic forbs from establishing and should be retained until a spring burn, just before you sow native forbs, so they can establish with less competition from exotic species.

In the, "Land of sleeping plains: Managing and restoring the native grasslands of south-eastern Australia" book released this year, Morgan says that burning to promote diversity should be done between mid-summer and the autumn break. He also acknowledges that burning at other times might be more effective for other purposes, such as combating exotic

annuals, and that timing should consider potential side-impacts, for example on the resident fauna. If burning to destroy or prevent seed production of particular exotic species the most effective time to burn would depend on the lifecycle of those species.

Ken Hodgkinson's burning trials with the Ginninderra Catchment Group also points to burning in autumn, but we don't have further details yet.

Even with this evidence suggesting burning in one season or the other under certain circumstances, there are other questions for which we don't know the answer. For example, how much of the native or exotic soil surface seed would be destroyed by burning in autumn or in spring? How well will native forb seedlings restrict exotic invasion if sown in autumn or in spring? Trialing burns in both seasons and monitoring the results may be the only way of finding the answers.

General Notes

- Whenever forb or grass seed is sown, the probability of successful establishment will be influenced by timing, seed quantity/quality, moisture conditions, and competition.
    - Even with a very high seeding rate ($1g.m^{-2}$ x 14 species), ideal biomass conditions (tussocks thinned, and litter removed), and ample watering, only 9 out of 14 sown species had emerged after 2 growth seasons (between early October sowing and the following May). Six out of those 9 species had < 0.5% seedling emergence.

- The term "under ideal conditions" used above implies suitable sowing practices with sufficient good seed and moisture, and without adverse conditions such as high nutrients, strong competition from other plants, grazing, waterlogging etc.

- Where there's a risk of exotic invasion, the likelihood can be reduced with active monitoring and control (spray, daub, remove, etc), but the time and effort needed could be large.

Appendix Two

PhD Exit Song

(To the tune of Jambalaya, by Hank Williams)

Goodbye Joe, me gotta go, me-o-my-o
All skilled up, what comes next is up to me now
Will I remember what I've learned, when I need-o
Has the journey just begun or just be-ended

Chorus
Well off we go to save the world, just in time-o
Cause mankind forgot to care 'bout the enviro
Counting frogs, planting seeds, every day-o
Son of a gun, we'll all get post-docs in Ohio

Research design, writing papers, supervisors
What a journey, wish I knew before I started
Fenner folk, come to the fore, when you need-o
In the end, it's about, what you learn-o

Thank-you folks, that's the show, me-o-my-o
No more fieldwork, stats, and asking "What's the question?"
Final thesis, when it's done, see you at Fellows
For one and all, it's my shout, drinks on me-o